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DOCUMENT-IDENTIFIER: US 5935927 A
TITLE: Compositions and methods for stimulating amyloid removal in amyloidogenic diseases using advanced glycosylation endproducts

US PATENT NO. (1):
5935927

Abstract Text (1):

The present invention relates generally to methods and compositions for treating amyloidogenic diseases such as Alzheimer's disease and the development of type II diabetes, in which deposition of amyloid in organs such as the brain and pancreas interfere with neurological function and insulin release, respectively. The methods and compositions are directed toward increasing the activity of scavenger cells within the body at recognizing and removing amyloid deposits from affected tissues and organs. Scavenger cells may be targeted to amyloid deposits by means of spontaneously-occurring chemical modifications called advanced glycosylation endproducts (AGEs). Compositions are described which increase scavenger cell activity towards AGE-modified amyloid. Amyloid removal may also be enhanced by increasing AGE levels in amyloid deposits within the body by administering AGE-modified amyloid targeting agents, which after becoming situated at sites containing amyloid, subsequently attract scavenger cells to degrade attendant amyloid. These methods and associated compositions result in a decrease in the extent of amyloid deposits in tissues, reducing the attendant pathology.

Brief Summary Text (2):

The present invention relates generally to the non-enzymatic glycosylation of amyloid proteins and the often consequent formation of advanced glycosylation endproducts (AGEs). Formation of AGE-amyloid can result in disease conditions or complications. The invention particularly relates to compositions and methods for the prevention and treatment of amyloidosis associated with neurodegenerative diseases, in particular Alzheimer's disease, and amyloidosis associated with Type II (adult onset) diabetes.

Brief Summary Text (4):

Amyloidosis and the .beta.-Amyloid Peptide

Brief Summary Text (5):

Amyloidosis generally refers to a physiological condition which involves deposition of insoluble polypeptides, termed amyloid polypeptides or amyloid proteins. There are a wide range of amyloid proteins found in various tissues throughout a subject, and a number of pathological conditions associated with various amyloidoses. For example, multiple myeloma can result in amyloidosis with the immunoglobulin proteins. Idiopathic familial Mediterranean fever also involves systemic amyloidosis. Perhaps the best known disease associated with amyloidosis is Alzheimer's disease.

Brief Summary Text (6):

Alzheimer's disease (AD) affects more than 30% of humans over 80 years of age, and as such, represents one of the most important health problems of developed countries (Evans et al., 1989, JAMA 262:2551-56; Katzman and Saitoh, 1991, FASEB J. 280:278-286). The etiology and pathogenesis of this progressive dementia is poorly understood, but symptomatic disease is associated with deposits of amyloid plaques, cerebrovascular amyloid and neurofibrillary tangles in the brain and cerebrovasculature. The number of plaques in AD patients' brains are typically 5- to-10 fold greater than in age-matched healthy controls. Increased levels of plaques may result from increased rate of synthesis of the components of the plaques, decreased rate of degradation, or some

combination of the two.

Brief Summary Text (7):

The primary protein component of plaques is the 42 amino acid (4.2 kDa) beta-Amyloid Peptide (.beta.AP), which derives from a family of larger Amyloid Peptide Precursor (APP) proteins (Glenner and Wong, 1984, Biochem. Biophys. Res. Commun. 120:885-890; Glenner and Wong, 1984, Biochem. Biophys. Res. Commun. 122:1131-35; Goidgaber et al., 1987, Science 235:8778-8780; Kang et al., 1987, Nature 325:733-736; Robakis et al., 1987, Proc. Natl. Acad. Sci. USA 84:4190-4194; Tanzi et al., 1987, Science 235:880-884). The process of amyloidosis is poorly understood, but requires at least .beta.AP. Recent evidence shows that .beta.AP may be found in extracellular spaces like cerebrospinal fluid (CSF) of the brain and conditioned media of many cell types. Since increased amounts of amyloid deposits are present in AD brains one simple hypothesis is that increased .beta.AP production leads to increased amyloidosis. Messenger RNAs encoding the APP precursors of .beta.AP increase about 2-fold in AD brains, which has suggested to some a possible 2-fold increase in rates of translation, which may explain increased amyloid plaque formation (e.g., Jacobsen et al., 1991, Neurobiol. Aging 12:585-592, and references cited therein; Palmert et al., 1989, Prog. Clin. Biol. Res. 317:971-984; Tanaka et al., 1990, Rinsho Byori 38:489-493; Tanaka et al., 1989, Biochem. Biophys. Res. Commun. 165:1406-1414). An example of an increased efficiency of .beta.AP production that correlates with increased plaque levels is found in a rare genetically linked familial form of Alzheimer's disease (Cai et al., 1992, Science 259:514-516; Citron et al., 1992, Nature 360:672-674; Mullan et al., 1992, Nature Genet. 1:345-347), known as a Swedish disease involving a double lysine-methionine (KM) to asparagine-leucine (NL) mutation in APP near the amino-terminus of .beta.AP. This mutation increases the release of extracellular .beta.AP in cultured cells. However, while this observation may partly explain amyloidosis in the Swedish disease (and Down's Syndrome), .beta.AP peptide levels in CSF of AD and healthy patients are the same (Oosawa et al., 1993, Soc. Neurosci. Abst. 19:1038; Shoji et al., 1992, Science 258:126129). Thus, although healthy subjects appear to possess similar quantities of .beta.AP as AD patients, they nevertheless fail to accumulate the high number and amount of amyloid plaques seen in their AD counterparts.

Brief Summary Text (9):

Aggregation of the components of amyloid is a critical step in the development of amyloidosis. Once formed, fibrillar aggregates of .beta.AP are extremely stable and not easily degraded. Amyloid plaques may be purified by their resistance to solubilization in boiling SDS and digestion with a variety of proteases. Additional treatment with 80% formic acid or 6M guanidine thiocyanate eventually solubilizes a portion of the plaque material. The solubilized protein is primarily the 42 amino acid .beta.AP. Yet even after these harsh denaturation treatments, dimers, tetramers and large molecular weight aggregates containing immunoreactive .beta.AP are found. This resistance to solubilization into soluble or monomeric components suggests extensive protein modifications.

Brief Summary Text (11):

In the absence of increased soluble .beta.AP in most cases of AD, the question remains how amyloid accumulates to a greater degree at different rates. Synthetic .beta.APs corresponding to the first 28, 40, or 42 amino acids of .beta.AP (i.e., .beta.AP 1-28, .beta.AP 1-40 and .beta.AP 1-42, respectively) display concentration-dependent aggregation kinetics in in vitro incubations. Fibrillar aggregates form in vitro and these appear similar to brain beta-amyloid fibrils at the morphological level using electron microscopy and at the light microscopy and spectroscopic levels using Congo Red and Thioflavin stains.

Brief Summary Text (15):

One common characteristic of Type II diabetics is the presence of amyloid plaques in the pancreas. Such plaques are found in 90% of Type II diabetics upon autopsy. As with Alzheimer's disease, the presence of amyloid plaques in the affected organ cannot be conclusively demonstrated until autopsy (see, Edgington, 1994, Bio/Technology 12:591). Two groups independently identified the major component of pancreatic amyloid plaques as a 37 amino acid polypeptide termed islet amyloid polypeptide (IAPP) (Westermarck et al., 1987, Proc. Natl. Acad. Sci. USA 84:3881-85; Westermarck et al., 1987, Am. J. Physiol. 127:414-417), or amylin (Cooper et al., 1987, Proc. Natl. Acad. Sci. USA 84:8628-32; Cooper et al., 1988, Proc. Natl. Acad. Sci. USA 85:7763-66); the peptides identified by both groups appear to be interchangeable (Amiel, 1993, Lancet 341:1249-50). In its soluble form, amylin antagonizes insulin, and thus appears to have a role in the regulation of bloodstream glucose levels (see, Edgington, supra).

Brief Summary Text (21):

Moreover, brown pigments with spectral and fluorescent properties similar to those of late-stage Maillard products have also been observed in vivo in association with several long-lived proteins, such as lens proteins and collagen from aged individuals. An age-related linear increase in pigment was observed in human dura collagen between the ages of 20 to 90 years (see Monnier and Cerami, 1981, Science 211:491-493; Monnier and Cerami, 1983, Biochem. Biophys. Acta 760:97-103; and Monnier et al., 1984, "Accelerated Age-Related Browning of Human Collagen in Diabetes Mellitus", Proc. Natl. Acad. Sci. USA 81:583-587). Interestingly, the aging of collagen can be mimicked in vitro in a much shorter period of time by crosslinking induced by incubation in solution with relatively high concentrations of glucose. The capture of other proteins and the formation of adducts by collagen, also noted, is theorized to occur by a crosslinking reaction, and is believed to account, for instance, for the observed accumulation of albumin and antibodies in kidney basement membrane (see Brownlee et al., 1983, J. Exp. Med. 158:1739-1744; and Kohn et al., 1984, Diabetes 33:57-59).

Brief Summary Text (26):

Based on their knowledge of the role of AGEs in disease, the present inventors have sought to identify factors that enhance aggregation of .beta.AP, and more importantly to identify agents and methods to inhibit the action of such factors and thus prevent amyloidosis, e.g., in Alzheimer's disease and other amyloid diseases. More particularly, the invention seeks to discover the relationship between advanced glycosylation endproduct formation and amyloidosis. Prior to the instant invention, there has been no appreciation of a relationship between amyloidosis and advanced glycosylation endproduct formation.

Brief Summary Text (30):

In particular, the inventors have discovered that AGE-amyloid polypeptides, in particular AGE-.beta. amyloid peptide (.beta.AP), facilitate further aggregation of amyloid polypeptides, whether such amyloid polypeptides are AGE-modified or not.

Brief Summary Text (32):

Thus, the invention relates to a method of modulating AGE-amyloid polypeptide-mediated amyloidosis in a mammal by controlling the formation of AGE-amyloid polypeptides. In a specific aspect of the invention, aggregation of .beta.AP and amylin have been determined to be enhanced by the glycosylation reaction of .beta.AP or amylin to form AGE-.beta.AP or AGE-amylin as defined herein. Accordingly, the invention particularly extends to a method for modulating the in vivo aggregation of .beta.AP and associated neurodegenerative amyloidosis by controlling the formation and presence of AGE-.beta.AP. The invention further particularly extends to a method for modulating the in vivo aggregation of amylin and associated pancreatic islet cell amyloidosis by controlling formation and presence of AGE-amylin.

Brief Summary Text (33):

It has also been discovered that individuals suffering from an amyloidogenic disease have more AGEs associated with the amyloid polypeptides that form the amyloid plaques characteristic of the disease. The presence and level of AGE-amyloid polypeptides may reflect the total body burden of amyloid polypeptides and their age. In particular, patients with Alzheimer's disease have more AGEs associated with .beta.AP than normal individuals of the same age, and patients with Type II diabetes may have more AGEs associated with amylin than normal individuals. Since the absolute levels of .beta.AP in AD and normal individuals is about the same, the presence of AGE-.beta.AP can be indicative or predictive of AD.

Brief Summary Text (34):

A corresponding diagnostic utility comprises the measurement of the course and extent of amyloidosis by a measurement of the presence and amount of AGE-amyloid polypeptides, and particularly AGE-.beta.AP and AGE-amylin, as defined herein. An assay is included that may use the AGE-amyloid polypeptide of the present invention to identify disease states characterized by the presence of the AGE-amyloid polypeptide. Additionally, such an assay can be utilized to monitor therapy and thus adjust a dosage regimen for a given disease state characterized by the presence of the AGE-amyloid polypeptide. In specific embodiments, the diagnostic assays of the invention may be used to monitor the presence or level of AGE-.beta.AP or AGE-amylin.

Brief Summary Text (35):

As noted above, AGE-amyloid polypeptide is useful as a marker of a variety of conditions in which the fluctuation in amyloid polypeptide levels may reflect the presence or onset of dysfunction or pathology. Moreover, AGE-amyloid polypeptide is

useful alone and in conjunction with known carriers and delivery vehicles, such as liposomes, for the transport of therapeutic and other agents, including in certain instances the AGE moieties themselves, across membranes and epithelial layers, for example, and particularly the blood brain barrier, to particular sites in a patient for treatment. The particular site of interest may be an amyloid plaque that recognizes an AGE-amyloid polypeptide, such as the AGE-.beta.AP, or AGE-amylin, or a portion thereof.

Brief Summary Text (36):

The presence of high levels of AGE-amyloid polypeptides in amyloidogenic diseases indicates that the normal clearance mechanisms for such polypeptides are faulty. Therefore, in a further aspect, the present invention provides compositions and methods for stimulating or inducing mechanisms of recognition and removal of AGE-amyloid in an animal, i.e., the invention contemplates activation of the scavenger system in an animal's body to remove the amyloid plaques. Such scavenger systems include the activity of phagocytic cells, e.g., macrophages and, in neural tissue, microglial cells.

Brief Summary Text (37):

Accordingly, the invention provides for stimulating or activating the natural scavenger systems by administration of stimulatory agents, including but not limited to, an advanced glycosylation endproduct, an AGE bound to a carrier, the fluorescent chromophore 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI) bound to a carrier, a monokine (e.g., lymphokine or cytokine) that stimulates phagocytic cells in the animal to increase the activity of recognizing and removing AGE-amyloid, and mixtures thereof. In a specific aspect, the AGE is an AGE-amyloid polypeptide.

Brief Summary Text (38):

Accordingly, the invention provides a method of preparing AGE-amyloid polypeptide, in particular AGE-.beta.AP or AGE-amylin, which comprises incubation with an advanced glycosylation endproduct or a compound which forms advanced glycosylation endproducts for a length of time sufficient to form said AGE-amyloid polypeptide, e.g., AGE-.beta.AP or AGE-amylin.

Brief Summary Text (39):

Pharmaceutical compositions are also disclosed that comprise an AGE-amyloid polypeptide in combination with a pharmaceutically acceptable carrier. Such pharmaceutical compositions may include an additional active agent(s) in some instances, and may be prepared and used for oral, parenteral or topical, e.g., transdermal, sublingual, buccal or transmucosal delivery. As stated, the pharmaceutical compositions can be in the form of a liposome in certain instances.

Brief Summary Text (40):

Generally, the therapeutic methods of the present invention contemplate the inhibition of in vivo amyloid aggregation by the administration of an agent or a pharmaceutical composition containing such agent or a plurality of such agents, for the inhibition of the formation of advanced glycosylation endproducts involving any or all of the amyloid polypeptide and amyloid precursor polypeptide, and materials subject to such in vivo aggregation. Such agents comprise antagonists of advanced glycosylation, and include antibodies to AGEs, antibodies to AGE-amyloid polypeptide, in particular AGE-.beta.AP and AGE-amylin, as well as other ligands that would bind and neutralize the foregoing antigens. Suitable agents may also be selected from those agents that are reactive with an active carbonyl moiety on an early glycosylation product, and preferably are selected from aminoguanidine, a-hydrazinohistidine, analogs of aminoguanidine, and pharmaceutical compositions containing any of the foregoing, all as recited in detail herein. The invention set forth herein contemplates the discovery of additional agents that may then be used in like fashion and for like purpose.

Brief Summary Text (41):

Accordingly, it is a principal object of the present invention to modulate and control the in vivo aggregation of amyloid polypeptides leading to amyloidosis by controlling the formation of advanced glycosylation endproducts (AGEs), and particularly AGEs involving such amyloid polypeptides.

Brief Summary Text (42):

It is a further object of the present invention to provide a method for the prognosis, monitoring, and/or diagnosis of conditions in which abnormal amyloid accumulation is a characteristic, by detecting and measuring the presence and extent of AGE-amyloid polypeptide formation.

Brief Summary Text (44):

It is a still further object of the present invention to provide a method for identifying new drugs and corresponding agents capable of treating abnormal amyloid polypeptide aggregation, in one aspect by use of an assay involving AGE-atmyloid polypeptide, in particular AGE-.beta.AP or AGE-amylin.

Brief Summary Text (45):

Still another object of the invention is to provide for removing amyloid plaques that have formed in a subject by activating the mechanisms for recognition and removal of AGE-amyloid in the body of a subject, and which may be directly or indirectly responsible for a pathology.

Brief Summary Text (46):

It is yet another object to utilize AGE-amyloid polypeptides, particularly AGE.beta.AP and AGE-amylin, to treat systemic or neurodegenerative diseases associated with amyloidosis, in particular Alzheimer's disease and Type II diabetes, respectively.

Brief Summary Text (47):

It is still a further object of the present invention to identify AGE-amyloid proteins and methods of inhibiting their formation in instances or disease conditions where the presence or biological activity of these AGE-amyloid proteins is detrimental to the host organism, or indicative of the presence of a disease state in the host organism.

Drawing Description Text (6):

FIG. 5 presents data showing that amyloid plaque-enriched fractions of Alzheimer's diseased pre-frontal cortex contain more AGE adducts per mg protein than equivalently prepared fractions of age-matched, non-demented controls. Each control patient is represented by a circle and AD patients by triangles. Each symbol represents the average of at least 4 independent measurements of immunoreactive AGE adducts for each patient sample with the mean of each patient group marked by a cross symbol.

Drawing Description Text (7):

FIGS. 6A-6C present photographs that demonstrate the co-localization of AGE and prion protein (PrP) in PrP associated lesions, which contain amyloid deposits, characteristic of the spongiform encephalopathy found in the neurodegenerative disease scrapie. Brain tissue sections were obtained from 300 day old hamsters intracranially infected with a strain of hamster scrapie, reacted with control or specific polyclonal rabbit antisera, followed by a second alkaline phosphatase-conjugated anti-rabbit antibody to detect rabbit antibodies. (6A) Rabbit anti-RNase at 1:500 dilution (control); (6B) Rabbit anti-PrP at 1:500 dilution; (6C) rabbit anti-AGE-RNase (Makita et al., 1992, J. Biol. Chem. 267:5133-38) at a 1:500 dilution. Note that similar structures are decorated by the rabbit antisera in (6B) and (6C).

Drawing Description Text (8):

FIGS. 7A-7D present absorption spectral data relating to association of a Thioflavin-T-Amadori product conjugate with fibrillar .beta.-amyloid peptide in vitro. (7A) Absorption spectrum of Thioflavin from 200 to 650nm after pelleting of fibrillar .beta.-amyloid peptide. (7B) Absorption spectrum of Thioflavin-T before pelleting. (7C) Absorption spectrum of dithionitrobenzene after pelleting. (7D) Absorption spectrum of dithionitrobenzene before pelleting.

Detailed Description Text (3):

In one aspect, the invention provides compositions and methods to prevent the formation or cross-linking of AGE-modified proteins involved in amyloidosis. In a particular embodiment, the invention relates to the prevention of amyloidosis of the .beta.-amyloid peptide (.beta.AP) by inhibiting the formation of advanced glycosylation endproduct (AGE)-modified .beta.AP. .beta.AP is a component of the amyloid plaques associated with Alzheimer's disease (AD), as well as other amyloidogenic degenerative neurological diseases. In another embodiment, the invention relates to the prevention of amyloidosis of amylin by inhibiting the formation of advanced glycosylation endproduct (AGE)-modified amylin. Amylin is a component of the amyloid fibrils found with pancreatic islet cells in association with Type II diabetes. In other embodiments, the invention relates to AGE-modulated amyloidosis of immunoglobulins produced by multiple myeloma, amyloidosis associated with serum amyloid A peptide, and amyloidosis of the protein associated with one of the various spongiform encephalopathies, i.e., prion protein (PrP) or scrapie-associated fibril (SAF) protein.

Detailed Description Text (4):

In another aspect, the invention provides for clearance of amyloid plaques by activating resident phagocytic cells that express AGE receptors, increasing the AGE content of the plaque, or both.

Detailed Description Text (5):

The invention is based, in part, on the discovery that the level of AGE found in brain samples from AD patients is significantly greater than in similarly prepared samples from age-matched control subjects. Additional evidence forming a basis, in part, for the invention is the observation that AGE epitopes are located in amyloid plaques in hamster-adapted murine scrapie, a form of spongiform encephalopathy. The invention is further based partially on experiments demonstrating that AGE-modification of .beta.AP enhances the efficiency of .beta.AP aggregation, and that an inhibitor of AGE formation, in particular, aminoguanidine (AG), can inhibit the AGE-enhanced aggregation of .beta.AP.

Detailed Description Text (7):

The terms "amyloid," "amyloid plaque," and "amyloid fibril" refer generally to insoluble proteinaceous substances with particular physical characteristics independent of the composition of proteins or other molecules that are found in the substance. Amyloid can be identified by its amorphous structure, eosinophilic staining, and homogeneous appearance. Protein or peptide components of amyloid are termed herein "amyloid polypeptides," and include, but are not limited to, .beta.AP; scrapie protein precursor or prion protein; immunoglobulin, including .kappa. or .lambda. light or heavy chains, or fragments thereof, produced by myelomas; serum amyloid A; .beta..sub.2-microglobulin; apoA1; gelsolin; cystatin C; (pro)calcitonin; atrial natriuretic factor; islet amyloid polypeptide, also known as amylin (see, Westermarck et al., 1987, Proc. Natl. Acad. Sci. USA 84:3881-85; Westermarck et al., 1987, Am. J. Physiol. 127:414-417; Cooper et al., 1987, Proc. Natl. Acad. Sci. USA 84:8628-32; Cooper et al., 1988, Proc. Natl. Acad. Sci. USA 85:7763-66; Amiel, 1993, Lancet 341:1249-50); and the like. It should be noted that human and cat amylin are amyloidogenic peptides, and aggregate spontaneously in vitro to form insoluble fibrils, whereas rat amylin, which differs from human amylin at six amino acid residues, is non-amyloidogenic and does not form fibrils (see, Lorenzo et al., 1994, Nature 368:756-760). In a specific aspect, the term "amyloid" is used herein to refer to substances that contain .beta.AP, scrapie protein, or amylin. "Amyloidosis" refers to the in vivo deposition or aggregation of proteins to form amyloid plaques or fibrils.

Detailed Description Text (8):

As used herein, the term "AGE-" refers to the compound which it modifies as the reaction product of either an advanced glycosylation endproduct or a compound which forms AGEs and the compound so modified, such as the .beta.AP or amylin moiety. AGE-amyloid polypeptide can be formed in vitro or in vivo by reacting an amyloidogenic polypeptide or amyloid as defined herein with an AGE, such as AGE-.beta.AP or AGE-amylin, or with a compound such as a reducing sugar, e.g., glucose, until the peptide is modified to form the AGE-peptide.

Detailed Description Text (9):

The term "glycosylation" is used herein to refer to the non-enzymatic reaction of reducing sugars with a nucleophile, in particular an amine group, on an amyloid polypeptide, such as .beta.AP, which leads to formation of AGEs. These processes are well known in the art, as described above. An alternative term for this process that has come more frequently into use is "glycation."

Detailed Description Text (10):

A molecule is "antigenic" when it is capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (antibody) or T cell antigen receptor. An antigenic peptide contains at least about and preferably at least about 10, amino acids. An antigenic portion of a molecule can be that portion that is immunodominant for antibody or T cell receptor recognition, or it can be a portion used to generate an antibody to the molecule by conjugating the antigenic portion to a larger, immunogenic carrier molecule for immunization. A molecule that is antigenic need not be itself immunogenic, i.e., capable of eliciting an immune response without coupling to such a carrier.

Detailed Description Text (14):

A disease or disorder is associated with amyloidosis when amyloid deposits or amyloid plaques are found in or in proximity to tissues affected by the disease, or when the disease is characterized by overproduction of a protein that is or can become insoluble. The amyloid plaques may provoke pathological effects directly or indirectly

by known or unknown mechanisms. Examples of amyloid diseases include, but are not limited to, systemic diseases, such as chronic inflammatory illnesses, multiple myeloma, macroglobulinemia, familial amyloid polyneuropathy (Portuguese) and cardiomyopathy (Danish), systemic senile amyloidosis, familial amyloid polyneuropathy (Iowa), familial amyloidosis (Finnish), Gerstmann-Straussler-Scheinker syndrome, familial amyloid nephropathy with urticaria and deafness (Muckle-Wells syndrome), medullary carcinoma of thyroid, isolated atrial amyloid, and hemodialysis-associated amyloidosis (HAA); and neurodegenerative diseases.

Detailed Description Text (15):

Type II diabetes is associated with amyloid fibrils or deposits of the pancreas, in particular the islet cells that produce insulin. As with Alzheimer's disease amyloid plaques, the amyloid plaques or fibrils provoke pathological effects. In particular, concentrations of human amylin at which fibrils form are toxic for human and rat pancreatic islet insulin-producing β -cells (Lorenzo et al., 1994, Nature 368:758-760). Accordingly, in a specific embodiment, the invention relates to Type II diabetic amyloidosis.

Detailed Description Text (16):

Chronic inflammatory illnesses, such as idiopathic familial Mediterranean fever, Muckle-Wells syndrome, chronic malarial infection, and the like, can result in expression of serum amyloid A, an acute phase protein which may undergo further processing and form amyloid deposits and plaques. For example, in the Third World, chronic malaria can lead to amyloidosis of the spleen and/or liver of an individual. The resulting organ failure can ultimately lead to death. Multiple myeloma is associated with overproduction of immunoglobulins, which immunoglobulins or fragments thereof can form amyloid deposits and plaques in organs or tissues in contact with the circulatory system. Deposition of transthyretin can result in familial amyloid polyneuropathy (Portuguese), familial amyloid cardiomyopathy (Danish), or systemic senile amyloidosis. Hemodialysis-associated amyloidosis is a complication among long-term hemodialysis patients, in which β_2 -microglobulin is a major protein constituent of the amyloid fibrils (Drueke, 1991, Miner. Electrolyte Metab. 17:261-272; Geyjo et al., 1985, Biochem. Biophys. Res. Commun. 129:701-706; Gorevic et al., 1986, Proc. Natl. Acad. Sci. USA 83:7908-12; Shirahama et al., 1985, Lab. Invest. 53:705-709).

Detailed Description Text (17):

As noted above, in addition to systemic amyloidosis, the present invention relates particularly to neurodegenerative diseases involving amyloidosis. The term "neurodegenerative disease" refers to a disease or disorder of the nervous system, particularly involving the brain, that manifests with symptoms characteristic of brain or nerve dysfunction, e.g., short-term or long-term memory lapse or defects, dementia, cognition defects, balance and coordination problems, and emotional and behavioral deficiencies. Such diseases are "associated with amyloidosis" when histopathological (biopsy) samples of brain tissue from subjects who demonstrate such symptoms would reveal amyloid plaque formation. As biopsy samples from brain, especially human brain, are obtained with great difficulty from living subjects or might not be available at all, often the association of a symptom or symptoms of neurodegenerative disease with amyloidosis is based on criteria other than the presence of amyloid deposits, such as plaques or fibrils, in a biopsy sample.

Detailed Description Text (18):

In a specific embodiment, according to the present invention the neurodegenerative disease associated with amyloidosis is Alzheimer's disease (AD). In other embodiments, the disease may be the rare Swedish disease characterized by a double KM to NL mutation in amyloid precursor protein (APP) near the amino-terminus of the β -AP portion of APP (Levy et al., 1990, Science 248:1124-26). Another such disease is hereditary cerebral hemorrhage with amyloidosis (HCHA or HCHWA)-Dutch type (Rozemuller et al., 1993, Am. J. Pathol. 142:1449-57; Roos et al., 1991, Ann. N.Y. Acad. Sci. 640:155-60; Timmers et al., 1990, Neurosci. Lett. 118:223-6; Haan et al., 1990, Arch. Neurol. 47:965-7). Other such diseases known in the art and within the scope of the present invention include, but are not limited to, sporadic cerebral amyloid angiopathy, hereditary cerebral amyloid angiopathy, Down's syndrome, Parkinson-dementia of Guam, and age-related asymptomatic amyloid angiopathy (see, e.g., Haan and Roos, 1990, Clin. Neurol. Neurosurg. 92:305-310; Glenner and Murphy, 1989, N. Neurol. Sci. 94:1-28; Frangione, 1989, Ann. Med. 21:69-72; Haan et al., 1992, Clin. Neuro. Neurosurg. 94:317-8; Fraser et al., 1992, Biochem. 31:10716-23; Coria et al., 1988, Lab. Invest. 58:454-8). The actual amino acid composition and size of the β -AP involved in each of these diseases may vary, as is known in the art (see above, said Wisniewski et al.,

1991, Biochem. Biophys. Res. Commun. 179:1247-54 and 1991, Biochem. Biophys. Res. Commun. 180:1528 [published erratum]; Prelli et al., 1990, Biochem. Biophys. Res. Commun. 170:301-307; Levy et al., 1990, Science 248:1124-26).

Detailed Description Text (22):

In one aspect, the present invention provides for therapeutic treatment for the prevention or inhibition of amyloidosis associated with diseases or disorders, e.g., neurodegenerative diseases, in particular Alzheimer's disease. In broad aspect, the therapeutic method of the invention involves administration of an agent that is capable of controlling the production, formation, or accumulation of advanced glycosylation endproducts. Such agents include, but are not limited to, antibodies against advanced glycosylation endproducts, ligands, including AGE receptors and active fragments thereof, capable of binding to and neutralizing advanced glycosylation endproducts, and compounds capable of inhibiting the formation of advanced glycosylation endproducts. In particular, the invention relates to an inhibitor of glycosylation, preferably an inhibitor of AGE formation, to the brain of a subject believed to be in need of such treatment. Such an agent is termed herein "capable of inhibiting the formation of AGEs", or alternatively an "inhibitor of AGE formation", "inhibitor of advanced glycosylation," or an "agent that inhibits advanced glycosylation."

Detailed Description Text (23):

The present invention further contemplates a dual therapeutic strategy, where agents that inhibit advanced glycosylation, such as aminoguanidine, may be administered to inhibit in vivo AGE-amyloid polypeptide formation and consequent initiation of amyloid polypeptide aggregation, plaque formation, and amyloidosis; and to react with any byproducts of an ongoing amyloid glycosylation to prevent reaction of these byproducts with proteins, particularly other amyloid polypeptides, resulting in proteolytically resistant cross-links in the amyloid plaque.

Detailed Description Text (24):

The therapeutic (and, as discussed below, the diagnostic) methods of the present invention contemplate the use of agents that have an impact on the formation of AGE-amyloid. Among these agents, antibodies to AGEs and other ligands may be prepared and used.

Detailed Description Text (25):

The rationale of the invention is to use agents which block the post-glycosylation step, i.e., the formation of fluorescent chromophores and/or molecular crosslinks whose presence is associated with, and leads to, the adverse sequelae of glycosylation. An ideal agent would prevent the formation of AGE-associated chromophores and/or cross-links bridging proteins and covalently trapping proteins onto other proteins, such as occurs in amyloid plaques.

Detailed Description Text (31):

In another embodiment, inhibitors of AGE can be antibodies. Antibodies can bind to and inactivate or mediate clearance of AGE-modified amyloid polypeptides. In one aspect of the invention, the antibody described in Makita et al. (1992, J. Biol. Chem. 267:5133-38) can be used. The invention further provides for generation of antibodies to AGE epitopes of AGE-amyloid polypeptides. Such antibodies can be prepared using techniques well known in the art. Preferably, the immunogen used to prepare the antibodies is an AGE-amyloid protein. In a specific aspect, AGE-.beta.AP can be used. In another embodiment, AGE-amylin can be used.

Detailed Description Text (32):

The AGE-.beta.AP or AGE-amylin may be used to produce antibody(ies) to themselves. Such antibodies can be produced and isolated by standard methods including the well known hybridoma techniques. Generally, antibodies can be produced by immunization of an animal with AGE-.beta.AP or AGE-amylin, free or conjugated with a carrier protein, such as but not limited to keyhole limpet hemocyanin (KLH) or BSA, preferably admixed with an adjuvant as defined above.

Detailed Description Text (33):

The term "antibody" includes any immunoglobulin, including antibodies and fragments thereof that binds a specific epitope, and such general definition is intended to apply herein. The term therefore encompasses polyclonal, monoclonal and chimeric antibodies, the last mentioned described in further detail in U.S. Pat. Nos. 4,816,397 and 4,816,567. Also, an "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically bind antigen.

Detailed Description Text (34):

Exemplary antibodies include antibody molecules such as intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the active binding site, including those portions known in the art as Fab, Fab', F(ab').sub.2 and F(v), which portions are preferred for use in therapeutic methods associated herein.

Detailed Description Text (35):

Fab and F(ab').sub.2 portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Pat. No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from F(ab').sub.2 portions by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide.

Detailed Description Text (36):

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. An antibody may be prepared having a plurality of antibody combining sites, each immunospecific for a different antigen, e.g., a bispecific (chimeric) antibody.

Detailed Description Text (38):

Preferably, the treatment is effected prophylactically, to prevent the initial formation of amyloid seed, which may facilitate aggregation of non-AGE modified amyloid polypeptide, such as soluble .beta.AP or soluble amylin, as well as AGE-amyloid polypeptide. In a particular embodiment, an inhibitor of AGE formation that can traverse the blood brain barrier, as described above, can be administered to a subject believed to be at increased risk for Alzheimer's disease or another neurodegenerative disease that involves amyloidosis to inhibit the onset or progression of the disease at an early stage. In another embodiment, the inhibitor of AGE formation can be injected intraperitoneally or intravenously in a subject believed to be at increase risk for Type II diabetes, e.g., a person suffering from obesity or one of the other conditions associated with Type II diabetes. For example, subjects that have a genetic predisposition to AD or Type II diabetes can be treated prophylactically. The actual dosage and treatment regimen for such prophylaxis can be readily determined by the ordinary skilled physician, taking into account the route of administration, age and weight of the patient, and the particular disease state for which the patient is undergoing treatment, as well as the stage thereof, and, of course, any side effects of the inhibitor, efficacy of the inhibitor, in accordance with customary medical procedures and practices.

Detailed Description Text (41):

In accordance with the present invention, a method and associated agents are disclosed for the inhibition and treatment of amyloidosis in animals by stimulating the bodies of such animals to increase their recognition of and affinity for advanced glycosylation endproducts. In particular, phagocytic cells such as monocytes, macrophages and/or microglial cells are treated with an agent capable of causing the phagocytic cells to increase their activity of recognizing and removing AGE-modified amyloid plaques.

Detailed Description Text (42):

The agents of the present invention comprise one or more stimulator compounds in turn, comprising a natural or synthetic advanced glycosylation endproduct alone or bound to a carrier, said carrier including a material selected from carbohydrates, proteins, synthetic polypeptides, lipids, bio-compatible natural and synthetic resins, antigens, and mixtures thereof. The stimulator compounds could include other advanced glycosylation endproducts that may be prepared from the reaction between sugars and other macromolecules, and monokines which stimulate phagocytic cells to increase their activity toward advanced glycosylation endproducts (see U.S. Pat. No. 4,900,747, issued Feb. 13, 1990 to Viassara et al., which is incorporated herein by reference in its entirety). Accordingly, the stimulator compound may comprise the compound FFI bound to a protein such as albumin. Alternately, the stimulator compound may comprise a synthetically derived advanced glycosylation endproduct which is prepared, for example, by the reaction of glucose or glucose-6-phosphate with albumin. This reaction product can be used alone or with a carrier in the same fashion as the FFI-albumin complex. In a specific aspect, the stimulator compound is an AGE-amyloid polypeptide.

Administration AGE-amyloid.

Detailed Description Text (45):

A further alternative embodiment of the method of the present invention and one which may be practiced independently or conjointly with the above recited method, is the ex vivo treatment of the phagocytic cells to expose them to the stimulator compounds. For example, a patient may be given an extracorporeal blood treatment in which blood is diverted out of the body from the arterial and venous system and is directed through a device which contains stimulator compounds and/or co-stimulatory agents which are suitably positioned to come in contact with the phagocytic cells within the blood. The stimulator compounds and/or co-stimulatory agents may be immobilized or may be allowed to enter the flow of the body fluid.

Detailed Description Text (47):

In a specific embodiment, AGE-modified amyloid polypeptides are useful for activating tissue phagocytic cells, such as macrophages, which can in turn metabolize amyloid deposits or plaques. Such an amyloid polypeptide may have an amino sequence identical to the native amino acid sequence, or it may be modified to include one or more additional sites for non-enzymatic glycation and AGE formation. For example, a histidine, asparagine, or glutamine residue (i.e., a polar or cationic residue) may be substituted with lysine.

Detailed Description Text (48):

As noted above, administration of an AGE-modified amyloid polypeptide can activate phagocytic mechanisms in tissue phagocytic cells. In addition to phagocytosing amyloid, such phagocytic cells may also secrete enzymes that help degrade amyloid, and may recruit other cells that can assist in the removal of amyloid.

Detailed Description Text (49):

In a further particular aspect, the AGE-.beta.AP or other AGE-neural amyloid polypeptides of this invention can be utilized as stimulants of activation of neural phagocytic cells, in particular microglia, to activate the microglia to effect removal of AGEs and/or AGE-modified polypeptides, and thus, amyloid. Such phagocytic cells are capable of recognizing and removing abnormal macromolecules by means of receptors on their surfaces which recognize specific chemical structures and bind them. Once the abnormal macromolecule is recognized in this way, the phagocytic cell may internalize the macromolecule and may then degrade it. In some instances, the phagocytic cell may in addition secrete enzymes and other factors to help degrade the molecule or particle extracellularly if it cannot be internalized or to induce other cells to participate in such degradation. After the amyloid is removed, normal function of the affected area may resume.

Detailed Description Text (50):

In another specific aspect, the invention contemplates administration of AGE-amylin to activate the body's absorption mechanisms to remove pancreatic amyloid plaques or fibrils associated with Type II diabetes.

Detailed Description Text (51):

The present invention contemplates that the phagocytic cells can be activated by exposure to stimulator compounds that potentiate the capability of these cells with respect to their recognition and affinity for, and capability to degrade, advanced glycosylation end products. In particular, the exposure of these cells to certain stimulator compounds has been found to increase the number of receptors developed on these cells and to thereby increase the capacity and efficiency of these cells with respect to the recognition and degradation of advanced glycosylation endproducts. Thus, in a specific aspect, the AGE-.beta.AP or AGE-amylin of the present invention can function as a stimulator compound, as can other compounds known to stimulate phagocyte-mediated AGE-specific activity (see U.S. Pat. Nos. 4,665,192, issued May 12, 1987 and No. 4,900,747, issued Feb. 13, 1990, and copending U.S. application Ser. No. 07/878,837, filed May 5, 1992).

Detailed Description Text (52):

Accordingly, the method of the present invention generally comprises exposing brain tissue to AGE-amyloid polypeptide, or exposing pancreatic tissue to AGE-amylin, which can result in activation of the mechanisms for an increase in the recognition and removal of amyloid that has undergone advanced glycosylation.

Detailed Description Text (53):

In a further embodiment, where a subject presently manifests the symptoms of a disease associated with amyloidosis as described above, particularly involving dementia in

neurodegeneration or adult onset diabetes, the present invention contemplates modifying the amyloid plaques to increase the level of AGEs, so as to increase the availability of the plaques as targets for degradation by the pathways of recognition and removal of AGE-modified molecules, in particular by activation of phagocytic cells. For example, amyloid targeting agents, like Congo Red and Thioflavin (see Caughey et al., U.S. Pat. No. 5,276,059, issued Jan. 4, 1994, which is hereby incorporated by reference in its entirety), including derivatives and analogs thereof that demonstrate affinity for binding amyloid, can be modified to bear AGEs or AGE precursors (hereinafter, both AGE and AGE precursor modified targeting agents are termed "AGE" modified targeting agents) and administered so as to target said AGEs or AGE precursors to amyloid deposits. The structures of Thioflavini T and Congo Red are shown below: ##STR4##

Detailed Description Text (54):

The AGE-amyloid targeting agents of the invention can be administered via any route, e.g., i.v., i.p., i.m., intraventricularly, intracranially, orally, nasally, through a skin patch, etc. In a specific aspect, these agents are modified to be capable of crossing the blood brain barrier, and thus modified, are attractive candidates for increasing the level of AGEs on amyloid plaques associated with neurodegenerative disease.

Detailed Description Text (55):

Any AGE or AGE precursor, such as an Amadori compound, can be conjugated to the amyloid targeting agent for use in increasing the level of AGE modification of amyloid. Examples of such AGEs or AGE precursors include, but are not limited to, FFI, fructopyranose and derivatives thereof, and the like. In specific embodiments, infra, Thioflavin is conjugated with an Amadori compound, such as 6-amino(1-deoxy-.beta.-D-fructopyranos-1-yl) and 6-N,N-dimethylamino(1-deoxy-.beta.-D-fructopyranos-1-yl) groups as shown in an Example, infra, to form AGE-Thioflavin (AGE-TF). In another embodiment, Congo Red is conjugated with an Amadori compound, e.g., as outlined in Scheme I, as follows: ##STR5##

Detailed Description Text (56):

The Thioflavin or Congo Red moiety targets the Amadori compound to amyloid plaques and fibrils, in order to induce AGE formation mediated by the Amadori product on or in immediate association with the amyloid plaque or fibril, resulting in formation of AGE-modified amyloid, thus increasing the likelihood of uptake of the amyloid plaque or fibril by local or recruited phagocytic cells.

Detailed Description Text (57):

In a specific embodiment, AGE-TF can be administered to an individual with AD in order to induce AGE formation in the .beta.AP-amyloid plaques associated with AD. Increased levels of AGE modification of the amyloid increases the likelihood of uptake by central nervous system microglia or recruited peripheral monocytes, or both, and facilitates removal of the amyloid.

Detailed Description Text (58):

In another specific embodiment, AGE-TF or AGE-Congo Red can be administered to an individual with Type II diabetes to induce AGE formation of the amylin-amyloid plaques associated with Type II diabetes. Increased levels of AGE modification of the amyloid increases the likelihood of uptake by local or recruited phagocytic cells, and facilitates removal of the amyloid.

Detailed Description Text (59):

The effectiveness of an AGE bearing targeting agent, such as AGE-TF or AGE-Congo Red, can be tested in vitro and in vivo for efficacy at AGE modification of amyloid. This AGE modification may be non-covalent through the association of the targeting agent and amyloid, or covalent due to the inherent reactivity of the AGE or AGE precursor for sites on the amyloid. In the following examples, the amyloid polypeptide .beta.AP and the amyloid targeting agent Thioflavin (TF) are described for convenience. However, the present disclosure contemplates performance of the same or similar assays with any amyloid model system and any amyloid-specific targeting agent, and is not intended to be limited to the following examples.

Detailed Description Text (60):

An in vitro assay can be used to determine the ability of AGE-TF to AGE modify insoluble or aggregated .beta.AP or amylin. For example, AGE-TF can be incubated with insoluble .beta.AP or insoluble amylin to produce AGE-modified insoluble or aggregated .beta.AP or amylin. The level of AGE modification can be determined, e.g., by ELISA using an anti-AGE antibody, and compared to a control treated with the unmodified

Thioflavin. Further testing for clearance of the AGE-modified insoluble .beta.AP can be conducted by incubation with cultured phagocytic cells, such as mouse peritoneal macrophages, elicited macrophages, the RAW 264.7 cell line, human peripheral monocytes, or microglia or astroglia primary cells or cell lines.

Detailed Description Text (61):

Involvement of AGE-receptor-mediated uptake by phagocytic cells can be evaluated with a standard binding assay. Insoluble or aggregated labelled .beta.AP (e.g., .sup.125 I-.beta.AP, although any labelling means known in the art, such as are discussed below, can be used) is contacted with an AGE-TF or TF alone (control), and incubated with about 10.sup.6 cells per well in the presence of increasing concentrations of cold AGE-BSA as a standard competitor. The amount of labelled .beta.AP or amylin bound in the absence of competing AGE-BSA is then compared to the amount in the presence of different concentrations of AGE-BSA. Standard methods can then be used to derive the number, affinity and association kinetics of AGE-modified amyloid binding sites on the cells. Labelling of the .beta.AP or amylin should precede AGE modification by AGE-TF treatment to ensure that the .beta.AP or amylin is the labelled moiety. Additional controls can include untreated .beta.AP or amylin alone.

Detailed Description Text (63):

The invention also contemplates use of in vivo assays to demonstrate AGE modification of amyloid. In one embodiment, AGE-TF or AGE-Congo Red can be administered to female Syrian hamsters, which develop amyloidosis in the liver, spleen and kidney within about one year after birth (resulting in a much shorter life expectancy than for male hamsters; see Coe and Ross, 1985, J. Clin. Invest. 76:66-74), and in which administration of diethylstilbestrol (DES) accelerates this amyloidosis. To determine the effect of AGE-TF or AGE-Congo Red, a group (n=4 to 10) of 6-12 month old hamsters treated with DES at 3 months of age are treated with 1-2 intraperitoneal injections of about 0.1 to about 1 mg of AGE-TF or AGE-Congo Red in an appropriate carrier, such as buffered saline, per week for varying periods of time. Control animals receive injections of the carrier, AGE alone, and TF or Congo Red alone. The presence and level of AGE-modification of amyloid deposits can be tested by the tissue squash method (Coe and Ross, 1990, J. Exp. Med. 171:1257-67) and ELISA (as described herein) after varying periods of time, e.g., 1 month, 2 months, and 4 months.

Detailed Description Text (64):

A similar in vivo assay can be performed on mice or hamsters inoculated intraperitoneally or intracranially with scrapie. About 1 month to 12 months, preferably 1 month to about 6 months, after infection with scrapie, the animals can be treated with AGE-TF or AGE-Congo Red, e.g., weekly or biweekly with intraperitoneal or intracranial injections of about 0.1 to about 1 mg of AGE-TF or AGE-Congo Red. Tissue samples or sections of affected organs (spleen for intraperitoneal infection, brain for intracranial infection) can be obtained. The presence of amyloid in spleen or brain can be detected histologically or immunochemically (immunohistologically or by ELISA with an anti-PrP antibody). The level of AGE modification of the tissue can be detected immunohistologically or by ELISA, e.g., using the anti-AGE-RNase antibody as described herein.

Detailed Description Text (65):

Such an experiment can also be performed with cats, rats, or mice that are genetically predisposed or treated to develop a Type II diabetic condition. About 1 month to 12 months, preferably 1 month to about 6 months, after diabetes onset, the animals can be treated with AGE-TF or AGE-Congo Red, e.g., weekly or biweekly with intraperitoneal or intravenous injections of about 0.1 to about 1 mg of AGE-TF or AGE-Congo Red. Tissue samples or sections of pancreas, particularly areas containing islet cells, can be obtained. The presence of amyloid can be detected histologically or immunochemically (immunohistologically or by ELISA). The level of AGE modification of the tissue can be detected immunohistologically or by ELISA, e.g., using the anti-AGE-RNase antibody as described herein.

Detailed Description Text (66):

The effectiveness of AGE modification of amyloid in inducing removal of the amyloid can be determined by detecting the amount of amyloid in affected tissues and comparing that amount to the amount in control animals after various periods of time. According to this aspect of the invention, the time course of pathology and treatment can involve amyloidosis, AGE-modification of amyloid, and clearance of the AGE-modified amyloid. Thus, the presence of amyloid and the level of AGE modification of the amyloid will depend on the point within the time course at which the sample is obtained for testing. The time course can be readily determined by obtaining and assaying samples at various

times.

Detailed Description Text (67):

In a further embodiment, the method of inducing AGE modification of the amyloid can be combined with the methods discussed above for activating mechanisms for the recognition and clearance of AGE-modified amyloid plaques.

Detailed Description Text (69):

The present invention also relates to a method for detecting the presence of or monitoring the course of a disease or disorder associated with amyloidosis comprising detecting the presence of or measuring the level or amount of an AGE-amyloid polypeptide that is found in the amyloid plaques characteristic of such a disease. Detecting the presence of AGE-amyloid polypeptides can indicate the existence of the disease condition, and thus may be useful alone or in conjunction with other criteria in diagnosis of such a disease. An increase in the level of AGE-amyloid polypeptide compared to the level detected in the subject at an earlier time, or to the level found in normal individuals, can indicate disease progression; a decrease in the level compared to the level in the subject at an earlier time, or the level found in normal individuals, can indicate regression of the disease.

Detailed Description Text (72):

The presence or level of AGEs may be followed directly by assay techniques such as those discussed herein, through the use of an appropriately labeled quantity of at least one of the binding partners to AGE-amyloid polypeptide as set forth herein. Alternately, AGEs can be used to raise binding partners or antagonists that could in turn, be labeled and introduced into a medium to test for the presence and amount of AGEs therein, and to thereby assess the state of the host from which the medium was drawn.

Detailed Description Text (73):

The term "ligands" includes such materials that would bind to AGE-amyloid peptide-binding partners, and would include such materials as are prepared by the reaction of AGE-.beta.AP or AGE-amylin with avidin or biotin, or the preparation of synthetic AGE-.beta.AP or AGE-amylin derivatives that may be prepared from the reaction of .beta.AP or amylin with reducing sugars such as glucose, glucose-6phosphate (G-6-P), fructose or ribose, and AGE-.beta.AP or AGE-amylin conjugation with peptides, proteins and other biochemicals such as bovine serum albumin (BSA), avidin, biotin derivatives, and enzymes such as alkaline phosphatase. Likewise, enzymes and other carriers that have undergone advanced glycosylation may also serve as ligands in any of the assays of the present invention. Accordingly, carriers such as carbohydrates, proteins, synthetic polypeptides, lipids and biocompatible natural and synthetic resins, and any mixtures of the same may be reacted with sugars to form advanced glycosylation endproducts and may thereby be useful in the present methods. The present diagnostic methods are intended to contemplate all of the foregoing materials within their scope.

Detailed Description Text (74):

The term "AGE binding partners" is intended to extend to anti-AGE antibodies and to other cellular AGE binding proteins or receptors for AGEs, which AGEs may be found on peptides, molecules and cells. A particular AGE binding partner is an anti-AGE antibody raised in rabbits and isolated therefrom for use as contemplated herein.

Detailed Description Text (80):

Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, .beta.-glucuronidase, .beta.-D-glucosidase, .beta.-D-galactosidase, urease, glucose oxidase plus peroxidase, hexokinase plus GPDase, RNase, glucose oxidase plus alkaline phosphatase, NAD oxidoreductase plus luciferase, phosphofructokinase plus phosphoenol pyruvate carboxylase, aspartate aminotransferase plus phosphoenol pyruvate decarboxylase, and alkaline phosphatase. U.S. Pat. Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternative labeling material and methods. A particular enzymatic detecting material is anti-rabbit antibody prepared in goats and conjugated with alkaline phosphatase through an isothiocyanate.

Detailed Description Text (81):

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine and auramine. A particular fluorescent detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

Detailed Description Text (82):

In addition to therapeutic uses, the antibodies of the invention can be used to detect AGE-amyloid polypeptides in amyloid or in solution. In particular, antibodies of the invention can be used to determine the amount and location of the AGE in amyloid plaques in the mammalian body. For convenience, the antibody(ies) to the AGE will be referred to herein as Ab.sub.1 and antibody(ies) reactive with Ab.sub.1 as Ab.sub.2.

Detailed Description Text (83):

The amount of AGE-amyloid polypeptide in a biological fluid or the degree of advanced glycosylation in amyloid plaques can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful utilize either the AGE-amyloid polypeptide labeled with a detectable label, antibody Ab.sub.1 labeled with a detectable label, or antibody Ab.sub.2 labeled with a detectable label. The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. An example of a "competitive" procedure is described in U.S. Pat. Nos. 3,654,090 and 3,850,752. An example of a "sandwich" procedure, is described in U.S. Pat. Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody", or "DASP" procedure.

Detailed Description Text (84):

In each instance, the AGE-modified substance forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed, and, if desired, the amount thereof, can be determined by known methods applicable to the detection of labels.

Detailed Description Text (85):

It will be seen from the above, that a characteristic property of Ab2 is that it will react with Ab.sub.1. This is because an antibody raised in the mammalian species in which Ab.sub.1 was raised has been used in another species as an antigen to raise the antibody Ab.sub.2. For example, Ab.sub.1 may be raised in rabbits and Ab.sub.2 may be raised in goats using a rabbit Ab as an antigen. Ab.sub.2 therefore would be anti-rabbit antibody raised in goats.

Detailed Description Text (86):

Accordingly, a test kit may be prepared for the demonstration of AGE-amyloid polypeptide in a sample, comprising:

Detailed Description Text (87):

(a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of AGE-amyloid polypeptide or an AGE binding partner to a detectable label;

Detailed Description Text (94):

By example, a solid phase assay system or kit may comprise the solid substrate with either bound binding partner and labeled AGE-amyloid polypeptide or bound AGE-amyloid polypeptide and labeled binding partner. A sample to be assayed is then placed in contact with the bound and unbound reagent. A competitive reaction between the labeled material and any unlabeled binding partner(s) in the sample will cause the retention of a dependent quantity of the former on the solid substrate, whereupon it can be precisely quantitatively identified. The foregoing explanation of a particular competitive assay system is presented herein for purposes of illustration only, in fulfillment of the duty to present an enabling disclosure of the invention. It is to be understood that the present invention contemplates a variety of diagnostic protocols within its spirit and scope.

Detailed Description Text (95):

In a preferred aspect of the invention, the AGE assay described in Makita et al. (1992, J. Biol. Chem. 267:5133-38) is used to detect the presence and determine the amount of AGE-amyloid in a tissue sample, particularly a sample that contains amyloid, or the amount of AGE-.beta.AP, AGE-scrapie protein, or AGE-amylin present in a sample.

Detailed Description Text (96):

In a specific embodiment, infra, antibodies reactive with AGEs are used to detect increased levels of AGE-proteins in brain tissue from individuals diagnosed with AD compared to normal individuals.

Detailed Description Text (97):

In another specific embodiment, antibodies to PrP (the scrapie or prion protein; e.g.,

Kascsak et al., 1987, J. Virol. 61:3688-3693) and antibodies to AGE can be used to show co-localization of these epitopes in a tissue sample. For example, brain sections from hamsters or mice infected with scrapie can be immunohistochemically stained with rabbit polyclonal anti-PrP, anti-AGE-RNase, and a control anti-RNase. In scrapie (a subacute spongiform encephalopathy), the characteristic spongiform encephalopathy is characterized by PrP-associated lesions, which contain amyloid deposits. These immunochemical studies can show that within a single scrapie diseased brain, PrP and AGEs co-localize in the amyloid of these lesions.

Detailed Description Text (100):
Age-amyloid in Alzheimer's Disease

Detailed Description Text (101):
 Alzheimer's disease (AD) is characterized by deposits of aggregated .beta.-amyloid peptide (.beta.AP) in the brain and cerebrovasculature. After a concentration-dependent lag period during in vitro incubations, soluble preparations of synthetic .beta.AP slowly form fibrillar aggregates that resemble natural amyloid and are measurable by sedimentation or Thioflavin-T-based fluorescence. Aggregation of soluble .beta.AP in these in vitro assays is enhanced by addition of small amounts of pre-aggregated .beta.-amyloid "seed" material. These seeds have also been prepared herein using a naturally occurring reaction between glucose and protein amino groups resulting in the formation of advanced glycosylation endproducts (AGEs) which chemically crosslink proteins. AGE-modified .beta.AP-nucleation seeds further accelerated aggregation of soluble .beta.AP compared to non-modified "seed" material. Over time, nonenzymatic advanced glycosylation also results in the gradual accumulation of a set of post-translational covalent adducts on long-lived proteins in vivo. Using a standardized competitive ELISA assay, plaque fractions of AD brains were found to contain about 3-fold more AGE adducts per mg protein than found in like preparations from healthy, age-matched controls. These results indicate that the in vivo half-life of .beta.-amyloid is prolonged in AD, resulting in greater accumulation of AGE modifications, which in turn can act to promote accumulation of additional amyloid.

Detailed Description Text (117):
 Although advanced glycosylation adducts form spontaneously in vivo, their accumulation is slow and becomes most notable with increasing time on long-lived tissue components. Along with time and the availability of susceptible protein amino groups, ambient glucose is the other major determinant of AGE formation. Thus, quantitative analysis of the degree of AGE modification of a single protein species under standardized glycemic conditions yields an index of the protein's half-life in vivo. It was found that plaque-enriched fractions isolated from AD brain samples contained about 3-fold more AGE modifications than did comparable fractions prepared from age-matched control brains, suggesting that .beta.AP half-life is prolonged in AD. That amyloid components exhibit a prolonged half-life in AD is also supported by studies that demonstrated the time-dependent, nonenzymatic isomerization of aspartyl-residues occurring at positions 1 and 7 of .beta.AP isolated from AD brain (Roher et al., 1993, J. Biol. Chem. 268:3072-3083), although this study latter did not include normal controls.

Detailed Description Text (118):
 AD is characterized by progressive dementia and increased numbers and amount of amyloid plaques compared to healthy age-matched controls. While a causal relationship between increased dementia and plaque numbers has not been proven, the gradual onset of symptoms appears to parallel the progressive deposition of .beta.-amyloid. From in vitro studies, it is clear that millimolar concentrations of soluble .beta.AP will spontaneously aggregate into fibrillar amyloid structures following a nucleation-dependent mechanism. At lower concentrations, the requirement for nucleus formation introduces a substantial lag period during which a solution of .beta.AP that still requires most of this time to form aggregation nuclei is indistinguishable from one on the verge of rapid aggregation and growth into a "one dimensional crystal" (Jarrett and Lansbury, 1993, Cell 73:1055-1058). The effect of this concentration-dependent nucleus formation is extreme as illustrated by published calculations that show an APP mutation in a Swedish form of familial AD which raises soluble .beta.AP concentrations 6-fold (Cai et al., 1992, Science 259:514-516; Citron et al., 1992, Nature 360:672-674) should reduce the lag time before aggregate growth occurs from 100 years to about 3 hours (Jarrett et al., 1993, Biochem. 32:4693-4697). Since the cerebrospinal fluid concentration of soluble .beta.AP in AD patients is the same as in age-matched controls (Oosawa et al., 1993, Soc. Neurosci. Abs. 19:1038; Shoji et al., 1992, Science 258:126, 129), the rate of concentration-dependent self-formation of nuclei, as reflected by the amounts of amyloid formed and deposited, might also be expected to be the same. As this latter similarity is not observed and AD

brains form substantially more deposits of aggregated .beta.AP than their non-diseased counterparts, then it appears that the increased amount of amyloid present in afflicted brain tissue results, at least in part, from more efficient nucleated aggregation than occurs in healthy brain parenchyma.

Detailed Description Text (124):

Hamsters were infected by intracerebral injection with a strain of hamster-adapted murine scrapie. After 300 days, the hamsters were sacrificed, the brains sectioned, and the sections fixed on microscope slides. The fixed sections were treated with 70% formic acid for 10 minutes and washed. The slides were then reacted with rabbit antisera specific for RNase (control antisera), prion protein (PrP; Kascsak et al., 1987, J. Virol. 61:3688-93), and AGE (anti-AGE-RNase antisera, as described in Makita et al., 1992, J. Biol. Chem. 267:5133-38). Each serum was diluted 1:500 prior to incubation with the tissue samples. The reactions were incubated overnight at 4.degree. C. Following reaction with the rabbit antisera, the samples were washed with PBS and reacted with an alkaline phosphatase (AP)-conjugated anti-rabbit antibody. The samples were developed with a fuschin AP substrate (Dako), which produces a red color.

Detailed Description Text (125):

The results of this experiment are shown in FIG. 6. The histological slides show regions of PrP-associated plaques identified with the anti-PrP antiserum (FIG. 6B). PrP is the purified scrapie protein that acts as the infectious agent, and that is associated with amyloid deposition in affected subjects. The anti-AGE antiserum generated against AGE-RNase also decorated the amyloid plaques. A control antiserum (anti-RNase) did not react with the histological samples.

Detailed Description Text (126):

These results indicate that the AGEs are present in the amyloid plaques that are characteristic of spongiform encephalopathy. As the scrapie amyloid plaque forms, it acquires AGE modifications that are detectable by antibodies. AGE modification of the scrapie amyloid plaque can occur through AGE modification of soluble PrP or the amyloid plaque itself, or by both mechanisms.

Detailed Description Text (142):

The present Example demonstrates that a modified Thioflavin-T, to which an Amadori product has been conjugated, co-precipitates with .beta.-amyloid in vitro. This observation demonstrates the feasibility of linking an Amadori product or AGE to Thioflavin-T for targeting to amyloid deposits.

Detailed Description Text (143):

A preparation of Compound 8, supra, was employed in this assay. Soluble .beta.-amyloid peptide was allowed to aggregate in the presence of phosphate buffer, Compound 8 or dithionitrobenzene and various compounds as described in Example 1, supra. The samples containing dithionitrobenzene are controls for non-specific association of a chromophoric small molecule with precipitated .beta.-amyloid peptide. An additional control sample was prepared containing phosphate buffer and the various compounds, but lacking .beta.-amyloid peptide. After overnight incubation, the tubes were centrifuged at 15,000.times.g for 30 minutes, half of the supernatant removed and added to a cuvette, and the absorbance spectrum of the supernatant from 200 to 600 nm obtained. In the absence of an association between compound 8 and aggregated sedimentable .beta.-amyloid components of the aggregated assay incubation, compound 8, which has an absorbance maximum at about 350 nm, will remain in the supernatant and its presence and concentration can be readily determined. Alternatively, association of Compound 8 with the precipitated .beta.-amyloid peptide would result in decreased absorbance at 350 nm. Thus, a decrease in the absorbance at 350 nm indicates that Compound 8 binds to fibrillar .beta.-amyloid that sediments during centrifugation under these conditions, as shown in Example 1, supra. In the control tubes, no decrease in the absorbance is expected, since the compound should remain soluble.

Detailed Description Text (144):

FIG. 7A shows that the absorbance at 350 nm of a supernatant solution containing Compound 8 is reduced when fibrillar .beta.-amyloid peptide present in the solution was pelleted, compared to the absorbance prior to pelleting (FIG. 7B). In contrast, the absorbance of dithionitrobenzene, which has not been reported to bind to aggregated .beta.-amyloid peptide deposits, does not decrease in the .beta.-amyloid peptide sample supernatant compared to the control (compare FIG. 7C--pellet formation--with 7D--no pellet formation). These data indicate that compound 8 specifically associates with insoluble, aggregated .beta.-amyloid peptide deposits.

Detailed Description Text (146):
Age-Amyloid in Type II Diabetes

Detailed Description Text (147):
Type II diabetes is characterized by deposits of aggregated amylin peptide in the pancreas. After a concentration-dependent lag period during in vitro incubations, soluble preparations of synthetic amylin slowly form fibrillar aggregates that resemble natural amyloid (Lorenzo et al., 1994, Nature 368:756-760) and are measurable by electron microscopy or by Congo Red birefringence under polarized light (Fraser et al., 1991, Biophys. J. 90:1194-1201). Aggregation of soluble amylin in these in vitro assays is expected to be enhanced by addition of small amounts of pre-aggregated amylin "seed" material. These seeds have also been prepared herein using a naturally occurring reaction between glucose and protein amino groups resulting in the formation of advanced glycosylation endproducts (AGEs) which chemically crosslink proteins. AGE-modified amylin-nucleation seeds are expected to further accelerate aggregation of soluble amylin compared to non-modified "seed" material. Over time, nonenzymatic advanced glycosylation, which is likely to occur at lysine-1 of amylin, and may occur at arginine-10, also results in the gradual accumulation of a set of post-translational covalent adducts on long-lived proteins in vivo. Using a standardized competitive ELISA assay, plaque fractions of Type II pancreatic islet cells are expected to be found to contain more AGE adducts per mg protein than found in like preparations from healthy, age-matched controls. These results indicate that the in vivo half-life of amylin is prolonged in Type II diabetes, resulting in greater accumulation of AGE modifications, which in turn can act to promote accumulation of additional amyloid.

Detailed Description Text (149):
Aggregation and Seeding Reactions. Synthetic, HPLC-purified peptides representing the 37 amino acid human or cat islet amyloid polypeptide or amylin may be obtained by synthesis or from a commercial source, such as Bachem (Torrance, Calif.) or Peninsula Laboratories. Quantitative aggregate formation with sub-millimolar amylin concentrations may be detected using the procedure of LeVine (1992, Protein Science 2:404-410). Briefly, fluorescence of aggregates added to 10 μ M Thioflavin-T (Aldrich)/50 mM potassium phosphate buffer, pH 6.0, can be measured upon excitation at 450.+-0.5 nm, and detection of emission at 482.+-0.10 nm on a Perkin Elmer LS-50B spectrofluorimeter. Alternatively, Congo red birefringence under polarized light can be used to detect aggregation (Fraser et al., supra). Small amounts of pre-formed aggregates or "nucleation seeds" are added to the soluble amylin and aggregation initiated, e.g., with 0.1M sodium acetate.

Detailed Description Text (152):
Measurement of AGEs with Competitive ELISA. Aliquots of pancreas containing amyloid fibrils from patients with and without Type II diabetes are resuspended in 2% sodium dodecylsulfate (SDS)/0.1M β -mercaptoethanol (ME), and Dounce homogenized. The homogenate is boiled for 10 minutes and then centrifuged at 10,000.times.g for 10 minutes. Supernatants are aspirated and the resulting pellets washed three times with PBS at 10,000.times.g for 10 minutes. This crude plaque fraction may be further washed twice with 4M urea and twice more with PBS before protease digestion. PBS-washed pellets are resuspended in PBS and 0.1% Proteinase-K (Boehringer Mannheim), digested overnight at 37.degree. C. and heat inactivated at 75.degree. C. for 3 hours. Quadruplicate aliquots of different amounts of plaque-containing pellet fractions are assayed for AGE content using a competitive ELISA (Makita et al., 1992, J. Biol. Chem. 267:5133-38), against standardized preparation of AGE-modified bovine serum albumin (AGE-BSA). Only values in the linear range of the standard curve should be included in the analyses.

Detailed Description Text (153):
Alternatively, tissue samples or amyloid fibril extracts can be prepared as described (Westermarck et al., 1986, Biochem. Biophys. Res. Commun. 140:827-831; Westermarck et al., 1987, Proc. Natl. Acad. Sci. USA 84:3881-85; Westermarck et al., 1987, Am. J. Physiol. 127:414-417; Cooper et al., 1987, Proc. Natl. Acad. Sci. USA 84:8628-32).

Detailed Description Text (156):
This experiment is expected to show that an AGE-amyloid seed comprising AGE-modified amylin will increase the rate of amylin aggregation. Similarly, soluble AGE-amylin is expected to aggregate more readily than soluble amylin. These results will indicate that, as discussed in Example 1, supra, with respect to β -AP aggregation in Alzheimer's disease, AGE-modification of amylin plays a role in pathogenic amyloidosis associated with Type II diabetes.

CLAIMS:

1. A method for enhancing removal of amyloid from a peripheral tissue of a mammal afflicted with or developing a disease or disorder associated with amyloidosis, comprising administering to said mammal an amyloid targeting agent conjugated with advanced glycosylation endproducts (AGEs) or AGE precursors.
2. The method of claim 1 wherein said amyloid targeting agent is selected from the group consisting of Congo Red and thioflavin.
5. A purified advanced glycosylation endproduct-modified (AGR)-amyloid polypeptide selected from the group consisting of AGE-amylin and AGE-serum amyloid A.
6. A pharmaceutical composition comprising the AGE-amyloid polypeptide of claim 5 and a pharmaceutically acceptable carrier.
7. An agent for enhancing removal of amyloid from tissue of a mammal comprising an amyloid targeting agent conjugated with advanced glycosylation endproducts (AGEs) or AGE precursors wherein said amyloid targeting agent is selected from the group consisting of Congo Red and thioflavin.

L2 ANSWER 3 OF 39 MEDLINE
 AN 2000236604 MEDLINE
 DN 20236604 PubMed ID: 10776846
 TI Anti-recombinant V antigen serum promotes uptake of *Yersinia enterocolitica* serotype 08 by macrophages.
 AU Roggenkamp A; Leitritz L; Sing A; Kempf V A; Baus K; Heesemann J
 CS Max von Pettenkofer-Institute for Hygiene and Microbiology, Ludwig Maximilians University Munich, Germany.. Rogge@m3401.mpk.med.uni-muenchen.de
 SO MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (1999 Dec) 188 (3) 151-9.
 Journal code: 0314524. ISSN: 0300-8584.
 CY GERMANY: Germany, Federal Republic of
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200005
 ED Entered STN: 20000525
 Last Updated on STN: 20000525
 Entered Medline: 20000518
 AB **Phagocytosis** resistance even in the presence of **opsonizing antibodies** is a key feature of pathogenic *Yersinia* spp. Nevertheless, **antibodies** against the secreted V **antigen** and the outer membrane protein YadA are known to mediate protection against *Y. enterocolitica* serotype 08 in a mouse model with intravenous infection. To investigate the impact of anti-V antigen serum on the interaction of *Y. enterocolitica* and phagocytic cells, gentamicin kill assays and immunofluorescence staining were performed. In contrast to anti-YadA, the presence of V antigen-specific antibodies resulted in an increased uptake of *yersiniae* by macrophages. The inhibition of phagocytosis by cytochalasin D suppressed the anti-V antigen-mediated uptake. The uptake-promoting effect of anti-V antigen was more distinct for macrophages than for polymorphonuclear leukocytes. The findings of the passive immunization experiments using an orogastric infection model were in agreement with those of cell-culture experiments. In the first 3 days of infection both antisera exhibit no protective effect on the multiplication of the bacteria in the Peyer's patches. Only mice passively immunized with anti-V antigen survived lethal oral infections with *Y. enterocolitica* serotype 08. Taken together, the results support the assumption that V antigen might be part of the translocation apparatus and that anti-V antigen inhibits the Yop translocation. In addition, antisera against in-frame-deleted recombinant V antigen were generated. Protection experiments using these antisera suggested that the type-specific region (amino acids 225-232) of the V antigen might not be a protective epitope.

L2 ANSWER 4 OF 39 MEDLINE
 AN 2000231768 MEDLINE
 DN 20231768 PubMed ID: 10768923
 TI Binding to and opsonophagocytic activity of O-antigen-specific monoclonal antibodies against encapsulated and nonencapsulated *Klebsiella pneumoniae* serotype O1 strains.
 AU Held T K; Jendrike N R; Rukavina T; Podschun R; Trautmann M
 CS Department of Hematology and Oncology, Charite/Campus Virchow-Klinikum, Humboldt University, 13353 Berlin, Germany.
 SO INFECTION AND IMMUNITY, (2000 May) 68 (5) 2402-9.
 Journal code: 0246127. ISSN: 0019-9567.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200006
 ED Entered STN: 20000622

Last Updated on STN: 20000622

Entered Medline: 20000613

- AB The high mortality of nosocomial infections caused by *Klebsiella* spp. has acted as a stimulus to develop immunotherapeutic approaches targeted against surface molecules of these bacteria. Since O-antigen-specific antibodies may add to the protective effect of K antisera, we tested the functional and binding capacity of O-antigen-specific monoclonal antibodies (MAbs) raised against different *Klebsiella* O antigens. The MAbs tested were specific for the O-polysaccharide partial antigens D-galactan II (MAB Ru-01), D-galactan I (MAB IV/4-5), or core oligosaccharide (MAB V/9-5) of the *Klebsiella* serogroup O1 antigen. In enzyme-linked immunosorbent assay binding experiments, we found that all MAbs recognized their epitopes on intact capsule-free bacteria; however, binding to encapsulated wild-type strains belonging to different K-antigen serotypes was significantly reduced. The K2 **antigen** acted as the strongest penetration barrier, while the K7 and K21 **antigens** allowed some, though diminished, **antibody** binding. In vitro **phagocytic** killing experiments showed that MAB Ru-01 possessed significant **opsonizing** activity for nonencapsulated O1 serogroup strains and also, to a much lesser extent, for encapsulated strains belonging to the O1:K7 and O1:K21 serotypes. MAbs or antisera specific for the D-galactan II antigen may thus be the most promising agents for further efforts to develop a second-generation *Klebsiella* hyperimmune globulin comprising both K- and O-antigen specificities.

L2 ANSWER 5 OF 39 MEDLINE

AN 1999392726 MEDLINE

DN 99392726 PubMed ID: 10465057

TI Flow cytometric analysis of IgG reactive to parasitized red blood cell membrane antigens in *Plasmodium falciparum*-immune individuals.

AU Drame I; Diouf B; Spiegel A; Garraud O; Perraut R

CS Unite d'Immunologie, Institut Pasteur de Dakar, Senegal.

SO ACTA TROPICA, (1999 Jul 30) 73 (2) 175-81.

Journal code: 0370374. ISSN: 0001-706X.

CY Netherlands

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199910

ED Entered STN: 19991014

Last Updated on STN: 19991014

Entered Medline: 19991004

- AB **Antigens** exposed at the surface of *Plasmodium falciparum* parasitized red blood cells (pRBCs) represent potential targets for protective **antibodies** involved in **opsonization** and immune **phagocytosis** of pRBCs. We measured the recognition of parasitized red blood cell membrane associated **antigens** by IgG in the plasma of clinically immune individuals by flow cytometry and ELISA. The plasmas were selected on the basis of preexisting IgG antibodies to pRBC membrane associated recombinant proteins. In every plasma sample IgG could bind the surface of live pRBCs in flow cytometry. In addition, there was a significant correlation between the level of IgG recognition of live pRBCs and of pRBC membrane ghost proteins or major identified antigens by ELISA. Flow cytometry thus represents a technique suitable to test for the accessibility and potential functionality of IgG antibodies directed to antigens expressed by the surface of pRBCs.

L2 ANSWER 6 OF 39 MEDLINE

AN 1999386854 MEDLINE

DN 99386854 PubMed ID: 10456908

TI The capsule of *Cryptococcus neoformans* reduces T-lymphocyte proliferation by reducing phagocytosis, which can be restored with anticapsular

antibody.

AU Syme R M; Bruno T F; Kozel T R; Mody C H
 CS Department of Microbiology and Infectious Diseases, University of Calgary,
 Calgary, Alberta, Canada T2N 4N1.

NC AI14209 (NIAID)
 SO INFECTION AND IMMUNITY, (1999 Sep) 67 (9) 4620-7.
 Journal code: 0246127. ISSN: 0019-9567.

CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199910
 ED Entered STN: 19991014
 Last Updated on STN: 19991014
 Entered Medline: 19991005

AB Cell-mediated immunity is critical for the host defense to *Cryptococcus* neoformans, as demonstrated by numerous animal studies and the prevalence of the infection in AIDS patients. Previous studies have established that the polysaccharide capsule contributes to the virulence of *C. neoformans* by suppressing T-lymphocyte proliferation, which reflects the clonal expansion of T lymphocytes that is a hallmark of cell-mediated immunity. The present studies were performed to identify the major mechanism by which polysaccharide impairs lymphocyte proliferation, since capsular polysaccharide has the potential to affect the development of T-lymphocyte responses by stimulating production of interleukin-10 (IL-10), inhibiting phagocytosis, and inducing shedding of cell surface receptors. We demonstrate that polysaccharide inhibits lymphocyte proliferation predominantly by blocking uptake of *C. neoformans*, which is crucial for subsequent lymphocyte proliferation. In addition, we show that polysaccharide did not suppress lymphocyte proliferation via an IL-10-dependent mechanism, nor did it affect critical surface receptor interactions on the T cell or **antigen-presenting cell**. Having established that polysaccharide impairs **phagocytosis**, we performed studies to determine whether **opsonization** with human serum or with anticapsular **antibody** could reverse this effect. Impaired uptake and lymphocyte proliferation that were induced by polysaccharide can be enhanced through opsonization with monoclonal antibodies or human serum, suggesting that antipolysaccharide antibodies might enhance the host defense by restoring uptake of the organism and subsequent presentation to T lymphocytes. These studies support the therapeutic potential of stimulating cell-mediated immunity to *C. neoformans* with anticapsular antibody.

L2 ANSWER 7 OF 39 MEDLINE
 AN 1998365860 MEDLINE
 DN 98365860 PubMed ID: 9700503
 TI Paradoxical behavior of asymmetric IgG antibodies.

AU Margni R A; Malan Borel I
 CS IDEHU-Instituto de Estudios de la Inmunidad Humoral, National Research Council of Argentina, University of Buenos Aires, Argentina..
 ramargni@ffyb.uba.ar

SO IMMUNOLOGICAL REVIEWS, (1998 Jun) 163 77-87. Ref: 61
 Journal code: 7702118. ISSN: 0105-2896.

CY Denmark
 DT Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
 LA English
 FS Priority Journals
 EM 199811
 ED Entered STN: 19990106
 Last Updated on STN: 19990106

Entered Medline: 19981106

AB

Changes in the quantity and quality of antibodies occur in the course of an immune response. This review describes the physicochemical and biological properties of asymmetric antibodies as well as their functions, beneficial or harmful to the host, according to the nature of the antigen and the particular situation in which they act. Asymmetric antibodies have two paratopes, one of high affinity, with K0 similar to that of symmetric antibodies, and the other one with an affinity for the antigen 100 times lower. Functional univalence is due to steric hindrance present in one of the paratopes by the carbohydrate moiety attached to the Fd fragment of the Fab region, so these **antibodies** are unable to form large **antibody-antigen** complexes and cannot trigger reactions, such as complement fixation, **phagocytic** activity and **antigen clearance**. When asymmetric IgG **antibodies** are specific for self-**antigens**, they prove beneficial for the host by exerting regulatory functions. In allergic manifestations, in autoimmune diseases and especially during pregnancy, despite the fact that the antigens responsible for the process are foreign to the host, they also perform beneficial activity. During pregnancy, the placenta secretes molecules or factors that regulate the synthesis of these antibodies, thus favoring fetal protection.

L2 ANSWER 8 OF 39 MEDLINE

AN 1998286564 MEDLINE

DN 98286564 PubMed ID: 9623361

TI Intravenous immunoglobulin preparations as immunomodulatory agents.

AU Dimitrijevic M; Popovic M; Stefanovic D; Petronijevic M

CS University School of Pharmacy, Belgrade.

SO VOJNOSANITETSKI PREGLED, (1998 Mar-Apr) 55 (2 Suppl) 63-9.

Journal code: 21530700R. ISSN: 0042-8450.

CY Yugoslavia

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199808

ED Entered STN: 19980903

Last Updated on STN: 19980903

Entered Medline: 19980824

AB

Certain immunopathologic conditions, such as hypersensitivity and autoimmune diseases, are characterized by quantitative and/or qualitative alterations of immune reactions. It is now believed that such immunologic disturbances generate from inadequate internal control and regulation of immunologic reactivity. Important member of the complex regulatory network that supervises an immune response are antibodies themselves. Since antibody is bifunctional molecule, its regulatory action deals with two separate molecular structures denoted as Fab and Fc portion. By Fab fragment, antibody interferes with the reaction with **antigens**, or participates in the regulatory idiotype/antiidiotype interactions. Fc-mediated regulation includes influence on complement activation cascade, formation and **clearance** of immune complexes, **phagocytosis**, ADCC activity, T- and B-cell function, cytokine profile, etc. In general, **antibody** triggers immune reactions, but also has the capacity to suppress them. Exogenous antibodies are likely to elicit similar effects on immune processes. Actually, it has been demonstrated that intravenously given immunoglobulins, particularly high-dose IgG, effectively combat harmful immune response in some chronic inflammatory and autoimmune disorders. Hence, preparations of immunoglobulins for intravenous use (IVIG) can be considered as an immunomodulatory agent. To achieve the property of modulating the immune response, IVIG products must contain intact (7S) IgG molecule and maintain sufficient concentrations in plasma.

L2 ANSWER 9 OF 39 MEDLINE
 AN 97275771 MEDLINE
 DN 97275771 PubMed ID: 9129541
 TI Immunogenicity and efficacy trials with Plasmodium falciparum recombinant antigens identified as targets of opsonizing antibodies in the naive squirrel monkey Saimiri sciureus.
 AU Perraut R; Mercereau-Puijalon O; Mattei D; Bourreau E; Bonnefoy S; Bonnemains B; Gysin J; Michel J C; da Silva L P
 CS Institut Pasteur de la Guyane Francaise, Cayenne, French Guiana.
 SO AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, (1997 Mar) 56 (3) 343-50.
 Journal code: 0370507. ISSN: 0002-9637.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Abridged Index Medicus Journals; Priority Journals
 EM 199705
 ED Entered STN: 19970514
 Last Updated on STN: 19970514
 Entered Medline: 19970508
 AB We have previously shown that Plasmodium falciparum recombinant antigens PfEB200, R23, and Pfi72 consistently inhibit opsonization of infected red blood cells by protective hyperimmune Saimiri sera, indicating that they present target epitopes involved in the phagocytosis of infected red blood cells. We report here an analysis of the immune response elicited in naive squirrel monkeys injected with the individual recombinant antigens or with a mixture of the three **antigens** combined with a synthetic peptide. In the three administration protocols investigated, there was no evidence for the production of **antibody** contributing to the **phagocytosis** of infected red blood cells, contrasting with the increase of **opsonizing antibodies** elicited by these **antigens** in monkeys with a prior (> or = 500 days) experience with malaria infection. However, the recombinant **antigens** were highly immunogenic, inducing specific antibody responses to P. falciparum and to the recombinant antigens. When the monkeys immunized with the antigen combination were challenged with blood-stage parasites, there was substantial protection: three of seven immunized animals self-cured and two others experienced a delayed peak of parasitemia. Taken together with our previous findings, these results suggest that PfEB200, R23, and Pfi72 constitute interesting vaccine candidates, and show that the presence of antibodies promoting phagocytosis of infected red blood cells is not a prerequisite for protection after immunization with these antigens in the Saimiri model.

L2 ANSWER 10 OF 39 MEDLINE
 AN 97149867 MEDLINE
 DN 97149867 PubMed ID: 8996661
 TI Immune response to exhaust gases derived from two-cycle combustion engine following experimental exposure.
 AU Reichrtova E; Bencko V
 CS Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic.
 SO CENTRAL EUROPEAN JOURNAL OF PUBLIC HEALTH, (1996 Feb) 4 (1) 7-10.
 Journal code: 9417324. ISSN: 1210-7778.
 CY Czech Republic
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199703
 ED Entered STN: 19970327
 Last Updated on STN: 19970327
 Entered Medline: 19970320

AB Experimental exposures using laboratory Wistar rats inhaling the exhaust gases derived from a two-cycle combustion engine under the chamber conditions have been carried out. Immune response reflecting a burden of the respiratory system in the exposed animals following the respiratory exposure has been investigated. In variously designed exposure experiments, the dynamics of pulmonary alveolar macrophages (PAM) activity to **phagocyte** particular **antigen** (sheep red blood cells), the hemolysis production, the total serum complement level and the capacity of the pulmonary **clearance** from the inhaled silica dust have been examined. The findings revealed the depressed ability to produce **antibodies** against sheep red blood cells (SRBC) in case of prolonged animals exposure during the time-interval of antibodies production. PAM **phagocytic** activity decreased considerably as well, in the first phases of the SRBC phagocytosis. The total serum complement level was found to be decreased in consonance with the decreased hemolysins production. The pulmonary clearance from the inhaled silica particles was statistically significantly elevated in the animals exposed long-term before the dusting only. In case of the prolonged exposure during the lung clearance period of 25 days, the exposed animals did not show the difference anymore, if compared to the non-exposed group.

L2 ANSWER 11 OF 39 MEDLINE

AN 96114482 MEDLINE

DN 96114482 PubMed ID: 8845060

TI Contradictory roles for antibody and complement in the interaction of *Brucella abortus* with its host.

AU Hoffmann E M; Houle J J

CS Department of Microbiology and Cell Science, University of Florida, Gainesville 32611-0700, USA.

SO CRITICAL REVIEWS IN MICROBIOLOGY, (1995) 21 (3) 153-63. Ref: 50
Journal code: 8914274. ISSN: 1040-841X.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

(REVIEW, TUTORIAL)

LA English

FS Priority Journals

EM 199610

ED Entered STN: 19961106

Last Updated on STN: 19961106

Entered Medline: 19961023

AB The ability of serum complement to kill bacteria has been linked to host resistance to Gram-negative bacteria. A mechanism for killing extracellular organisms during early invasion, following release from infected phagocytic cells, or during bacteremia would contribute to a host's ability to resist disease. In fact, the ability of serum complement to kill bacteria has been linked to disease resistance. *Brucella abortus* are Gram-negative intracellular pathogens. Resistance to these bacteria involves the coordinated activities of the cellular and humoral immune systems. The existence of serum-resistant forms of *B. abortus* has been established, and it has been shown that these bacteria can resist the killing action of complement even in the presence of specific antibody. Antibody is usually necessary for complement-mediated killing of smooth (virulent) forms of Gram-negative bacteria. An anomalous situation exists with some isolates of smooth *B. abortus*. Sera containing high titers of specific antibody do not support killing unless they are diluted. In the bovine, this phenomenon is associated with IgG1 and IgG2 antibodies. This finding may account for the lack of positive correlation between antibody levels and resistance to disease, which has led, perhaps wrongly, to the idea that antibody and complement are not important in resistance to brucellosis. Available evidence suggests that antibody may have contradictory roles in the interactions between a host and bacteria.

Avirulent (rough) forms of the organism would be rapidly killed by complement shortly after invasion, but serum-resistant smooth forms of the organism would survive and invade resident phagocytic cells. During the process of invasion and phagocytosis, the bacteria would initiate an immune response. With time, some *B. abortus* organisms would be released from infected phagocytic cells. In the early stages of this process, the bacteria would encounter IgM antibody and low concentrations of IgG antibody. These would cause complement-mediated killing, and infection would be restricted to resident **phagocytic** cells. However, the immune response to *B. abortus* **antigens** would be intensified, and IgG antibody levels would increase. High concentrations of **antibody** do no support complement-mediated killing of extracellular *B. abortus*, but the bacteria would be **opsonized** by antibody and complement component fragments. This would lead to increased **phagocytosis** of extracellular *B. abortus* as they appear, and concomitant extension of disease. Because of high levels of antibody would block complement-mediated killing of *B. abortus*, resistance to disease at this point would be dependent on cell-mediated immunity.

L2 ANSWER 12 OF 39 MEDLINE
 AN 95115528 MEDLINE
 DN 95115528 PubMed ID: 7815919
 TI Phenotypic variability of X-protein expression by mastitis-causing *Streptococcus agalactiae* of serotype NT/X and opsonic activities of specific antibodies.
 AU Rainard P; Sarradin P; Poutrel B
 CS Laboratoire de Pathologie Infectieuse et d'Immunologie, Centre de Recherches de Tours, Nouzilly, France.
 SO MICROBIAL PATHOGENESIS, (1994 May) 16 (5) 359-72.
 Journal code: 8606191. ISSN: 0882-4010.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199502
 ED Entered STN: 19950217
 Last Updated on STN: 19950217
 Entered Medline: 19950208
 AB This study examined the role of **antibodies** against the X-protein, a surface-localized **antigen** frequently associated with streptococci causing mastitis in cattle, in the **opsonization** and **phagocytosis** of unencapsulated *Streptococcus agalactiae*. The analysis of various strains of serotype NT/X by flow cytometry, after labeling with a monoclonal antibody to X-protein, revealed that they consisted of a mixture of unstained and stained bacteria. Cloning of mother strains yielded clones of unstained bacteria but not homogeneous clones of stained bacteria. Analysis by ELISA of an unstained clone (4.1) derived from the reference NT/X strain 24/60 indicated that it expressed low amount of X-protein at its surface, about 25 times less than the stained clone 24/60 5.6. Colloidal gold immunolabeling showed the X-protein at the periphery of bacteria (of clone 5.6 and in lower amount of clone 4.1), at a distance from the cell wall. Bovine antibodies (essentially IgG) to X-protein behaved like the monoclonal antibody in the cytometric assay. They activated the classical pathway of complement as shown by the deposition of C1q and C4 on bacteria. Deposition of C4 also occurred on the low-surface-producing clone 4.1 in the presence of antibodies to X-protein, although less efficiently than on the high-surface-producing clone 5.6. When used alone, antibodies promoted the ingestion of bacteria and heat-inactivated immune serum promoted the chemiluminescence activity and the killing by polymorphonuclear cells. In conclusion, antibodies to X-protein induced the deposition of C3 by the classical pathway and were also able to stimulate opsonophagocytic killing

of X-bearing S. agalactiae in the absence of deposited C3.

L2 ANSWER 13 OF 39 MEDLINE
AN 95037613 MEDLINE
DN 95037613 PubMed ID: 7950290
TI Circulatory clearance of transfused antibody-sensitized red cells in an entirely allogenic rabbit model.
AU Faust A; Kissel K; Neppert J
CS Institut für Klinische Immunologie und Transfusionsmedizin, Justus-Liebig-Universität, Giessen.
SO INFUSIONSTHERAPIE UND TRANSFUSIONSMEDIZIN, (1994 Aug) 21 (4) 260-4. Journal code: 9209406. ISSN: 1019-8466.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199412
ED Entered STN: 19950110
Last Updated on STN: 19950110
Entered Medline: 19941223
AB BACKGROUND: Antibodies against histocompatibility antigens on phagocytes abrogate their Fc gamma-receptor-mediated functions in vitro. Studies were carried out to determine whether this phenomenon also exists in vivo. METHODS: An allogenic blood group antibody was generated in rabbits. Red cells sensitized with this antibody were internalized in vitro by rabbit phagocytes. Another allogenic antibody specific for major histocompatibility complex (MHC) antigens of rabbits was produced. Plasma containing this antibody was infused into rabbits with **phagocytes** expressing the corresponding MHC **antigens**. Thereafter, the sensitized rabbit red blood cells were transfused into the rabbits. RESULTS: The following 3 phases of the circulatory **clearance** of transfused sensitized red cells could be observed when MHC **antibodies** were not given prior to the transfusion: 1. initial rapid clearance of the cells ($t/2 = 1.7-3.3$ min), 2. release of the cells back into the circulation after 0.5-24 h and 3. terminal slow clearance which was, however, faster than that with unsensitized cells. Two independent experiments carried out on the same recipient out of the 3 recipients analysed showed that the prior treatment of the recipient with MHC-alloantibodies extended the circulatory half-life of the sensitized red cells during the terminal phase from $t/2 = 1$ day to $t/2 = 3$ and 4 days, respectively. CONCLUSION: Antibodies against MHC antigens on phagocytes can reduce the Fc gamma-receptor-mediated immune elimination of sensitized red cells. This corresponds to the observation that many cases of morbus haemolyticus neonatorum show unexpectedly reduced levels of foetal red cell destruction and a good clinical outcome if mothers not only produce antibodies to Rh (D) but also to foetal MHC antigens.

L2 ANSWER 14 OF 39 MEDLINE
AN 94301030 MEDLINE
DN 94301030 PubMed ID: 8028388
TI Adhesion molecules in lung diseases.
AU Hamacher J; Schaberg T
CS Department of Pulmonary Medicine, Chest Hospital Heckeshorn-Zehlendorf Zum Heckeshorn, Berlin, Germany.
SO LUNG, (1994) 172 (4) 189-213. Ref: 148 Journal code: 7701875. ISSN: 0341-2040.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA English
FS Priority Journals

EM 199408
ED Entered STN: 19940818
Last Updated on STN: 19940818
Entered Medline: 19940809

AB The human body possesses highly specialized cellular defense mechanisms that, when activated pathologically, can induce a number of immunologic disorders. For a normal cellular immune response, the following conditions must be fulfilled: (1) accumulation of white blood cells, (2) their diapedesis through the vessel walls of the inflammatory area affected by an injurious agent, and (3) normal cellular effector functions in the tissue. This cascade of inflammatory processes has recently been shown to be regulated by a group of molecules that are termed adhesion molecules and consist of three subfamilies: selectins, the immunoglobulin supergene family, and integrins. The cellular functions influenced by adhesion molecules include, among others, cytotoxic T-cell responses, CD4-dependent activation of B lymphocytes by T lymphocytes, activation of granulocytes and macrophages, **phagocytosis** of **opsonized** particles by monocytes, macrophages, and granulocytes, **antigen**-presenting function of macrophages, their **antibody**-dependent cytotoxicity, initiation of a respiratory burst by white blood cells, and activation of fibroblasts. Studies performed in recent years have shown that pathogenetically relevant changes in the expression and function of adhesion molecules are involved in a variety of pulmonary diseases. These changes include the accumulation and activation of alveolar macrophages in smokers, experimentally induced bronchial hyperreactivity in bronchial asthma, accumulation of eosinophils in allergic rhinitis, bleomycin-induced pulmonary fibrosis, binding of viruses and bacteria to respiratory mucosa, and various mechanisms of acute damage to pulmonary parenchyma. Though their role in tumor development is still unclear, adhesion molecules are obviously involved in determining the route and organotropism of metastases. Further studies of the function of adhesion molecules in pulmonary diseases will contribute to our understanding of the pathomechanisms of these diseases and, through the development of specific antibodies, may provide attractive new therapeutic approaches to problems for which treatment is not yet available.

L2 ANSWER 15 OF 39 MEDLINE
AN 94296015 MEDLINE
DN 94296015 PubMed ID: 8024214
TI Immune response to Moraxella catarrhalis in children with otitis media: opsonophagocytosis with antigen-coated latex beads.
AU Faden H; Hong J J; Pahade N
CS Department of Pediatrics, State University of New York, Buffalo School of Medicine.
NC 19679
28304
SO ANNALS OF OTOTOLOGY, RHINOLOGY AND LARYNGOLOGY, (1994 Jul) 103 (7) 522-4.
Journal code: 0407300. ISSN: 0003-4894.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199408
ED Entered STN: 19940815
Last Updated on STN: 19940815
Entered Medline: 19940802

AB Opsonic antibody activity against Moraxella catarrhalis was determined in sera from children with otitis media. The **antibody** was determined with a new assay utilizing outer membrane **antigen** -coated latex beads. **Antigen**-coated beads **opsonized** in heat-inactivated pooled human serum **phagocytosed** 47.5 +/- 36.1 beads per 100 neutrophils compared to 15.6 +/- 10.2 beads per 100

neutrophils opsonized in hypogammaglobulinemic serum ($p < .025$).
Antigen-coated beads opsonized in homologous sera from 11 children with M. catarrhalis otitis media demonstrated increased opsonic activity in convalescent sera (34.6 ± 27.1) compared to acute sera (15.5 ± 6.7 ; $p < .05$). These data suggest that infection with M catarrhalis is associated with the development of opsonic antibody.

L2 ANSWER 16 OF 39 MEDLINE
AN 94089225 MEDLINE
DN 94089225 PubMed ID: 8265200
TI K-antigens in Porphyromonas gingivalis are associated with virulence.
AU van Winkelhoff A J; Appelmek B J; Kippuw N; de Graaff J
CS Department of Oral Microbiology, Academic Centre for Dentistry Amsterdam (ACTA), The Netherlands.
SO ORAL MICROBIOLOGY AND IMMUNOLOGY, (1993 Oct) 8 (5) 259-65.
Journal code: 8707451. ISSN: 0902-0055.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Dental Journals
EM 199401
ED Entered STN: 19940209
Last Updated on STN: 19940209
Entered Medline: 19940127
AB We investigated antigens in spreading and non-spreading Porphyromonas gingivalis strains. On the basis of differences in virulence in the mouse model, 8 strains were selected for antiserum production in rabbits. Hyperimmune sera were tested by double immunoprecipitation and immunoelectrophoresis. Besides a common antigen, differences in antigenic composition were observed in the thermolabile antigens between all strains tested. Two different heat-stable antigens were found after heating at 120 degrees C. One such antigen was detected after sonication of the pellet fraction of autoclaved P. gingivalis cells. This antigen cross-reacted with 6 of the 8 immune sera. This somatic antigen was almost neutrally charged and sensitive to sodium periodate treatment, suggestive of lipopolysaccharide. A second heat-stable antigen was detected in the supernatant of autoclaved strains of W83, W50, HG184 and A7A1-28. These non-somatic antigens were strain-specific, i.e., no cross-reactivity was found with heterologous hyperimmune sera. An exception was strain W50, which had a non-somatic heat-stable antigen which was recognized by W83 antiserum. These antigens were resistant to DNase, RNase and proteinase-K treatment but were degraded by sodium periodate. In immunoelectrophoresis, these antigens appeared to be negatively charged. These properties are characteristics of a K-antigen, which likely represent a thermostable carbohydrate capsule. The presence of K-antigen correlates very well with the serum resistance, the low chemiluminescence, the resistance to phagocytosis and the need for opsonization with specific antibodies for complement-mediated killing of virulent P. gingivalis strains. (ABSTRACT TRUNCATED AT 250 WORDS)

L2 ANSWER 17 OF 39 MEDLINE
AN 93242611 MEDLINE
DN 93242611 PubMed ID: 8480333
TI Acute inflammatory response to sheep red blood cell challenge in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): phenotypic and functional analysis of peritoneal exudate cells.
AU Kerkvliet N I; Oughton J A
CS College of Veterinary Medicine, Oregon State University, Corvallis 97331.
NC 1 R01 ES 03966 (NIEHS)
ES-00210 (NIEHS)
SO TOXICOLOGY AND APPLIED PHARMACOLOGY, (1993 Apr) 119 (2) 248-57.
Journal code: 0416575. ISSN: 0041-008X.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199305
ED Entered STN: 19930611

Last Updated on STN: 19930611
Entered Medline: 19930525

AB TCDD is a widespread environmental contaminant of concern to human health because of its well-recognized immunotoxicity in laboratory animals. Suppression of the murine antibody response to xenogeneic erythrocytes has been shown to be one of the most sensitive assays for TCDD immunotoxicity. However, the cellular mechanisms underlying the suppressed immune function have not been fully elucidated. In the present studies, peritoneal macrophage recruitment, activation, and antigen-presenting function in response to sheep red blood cell (SRBC) injection were compared in C57Bl/6 mice treated with a single oral dose of 0 or 5 micrograms TCDD/kg. In vehicle-treated mice, SRBC injection induced a typical inflammatory response in the peritoneal cavity. Within 6 hr, the number of neutrophils increased and remained elevated until 40 hr. Macrophage numbers increased at 24 hr and remained elevated through 72 hr. In TCDD-treated mice, a hyperinflammatory response to SRBC was observed. The total number of peritoneal exudate cells was significantly greater at 16, 24, and 40 hr after SRBC challenge when compared to that of vehicle-treated mice. The increased number of peritoneal cells reflected significant increases in both neutrophils and macrophages. Mac-1+ peritoneal cells were examined by two-color flow cytometric analysis on Days 0-3 after SRBC injection for expression of the activation markers F4/80 and I-A. The intensity of F4/80 fluorescence significantly decreased 24-72 hr following SRBC challenge, while fluorescence associated with I-A significantly increased at 72 hr. These changes are consistent with macrophage activation. TCDD did not significantly alter F4/80 expression on Mac-1+ cells, whereas I-A expression was increased earlier on cells from TCDD-treated mice. However, TCDD treatment did not alter the antigen presentation function of peritoneal cells, assessed by their ability to induce the proliferation of SRBC-primed T cells in vitro. The antigen-presenting function of adherent spleen cells was also not altered by TCDD exposure. To test the hypothesis that an excess number of **phagocytes** in TCDD-treated mice were **clearing** the **antigen** more efficiently, leading to a smaller (e.g., suppressed) **antibody** response, we attempted to overcome TCDD suppression by increasing the amount of SRBC **antigen** used for challenge. However, the magnitude of the anti-SRBC response in TCDD-treated mice was not significantly altered by increasing the antigen challenge dose, suggesting that enhanced clearance of antigen by macrophage is not a mechanism for TCDD-induced suppression of the anti-SRBC response. (ABSTRACT TRUNCATED AT 400 WORDS)

L2 ANSWER 18 OF 39 MEDLINE
AN 90331743 MEDLINE
DN 90331743 PubMed ID: 2640484
TI [Asymmetric IgG antibodies. Structural, immunochemical and biological studies].
Anticuerpos IgG asimetricos. Estudios estructurales, inmunoquimicos y biologicos.
AU Margni R A
SO MEDICINA, (1989) 49 (2) 147-54.
Journal code: 0204271. ISSN: 0025-7680.
CY Argentina
DT Journal; Article; (JOURNAL ARTICLE)
LA Spanish
FS Priority Journals
EM 199009

ED Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900904
AB Studies of our laboratory have shown that the coprecipitating antibodies are IgG molecules which possess an asymmetric structure due to a carbohydrate group present in only one of the Fab regions of the molecule. The combination of the corresponding antibody site with antigen is sterically hindered by the carbohydrate group, and as a consequence, the molecule is functionally univalent. As no aggregation of the **antibody** takes place, effector immune mechanisms such as complement fixation, **phagocytosis**, **clearance** of **antigen**, etc. are not triggered. However, since nonprecipitating **antibodies** can firmly combine with **antigen**, they act in a competitive way when they are mixed with precipitating antibodies of the same specificity. It has been demonstrated that hybridomas synthesize both symmetric and asymmetric molecules indicating that precipitating and nonprecipitating antibodies are elaborated by the same cellular clone. Nonprecipitating antibodies do not precipitate the antigen but they are able to agglutinate sheep red blood cells sensitized with the specific antigen. Because red blood cells of sheep and of many other animal species possess a membrane receptor for aggregated Fc, agglutination of sensitized sheep red cells by nonprecipitating antibodies is a consequence of a mixed mechanism in which one Fab and the Fc fragment of the IgG molecule participate. In animals immunized with soluble antigens, the nonprecipitating antibody represents 10-15% of the total. Higher concentrations have been observed when the inoculated antigen is particulate. In cows infected by B. aortus and in humans infected by T. cruzi, the percentage of nonprecipitating antibody ranges between 30-70% of the total antibodies. (ABSTRACT TRUNCATED AT 250 WORDS)

L2 ANSWER 19 OF 39 MEDLINE
AN 88189327 MEDLINE
DN 88189327 PubMed ID: 3258649
TI The binding site for Clq on IgG.
AU Duncan A R; Winter G
CS MRC Laboratory of Molecular Biology, Cambridge, UK.
SO NATURE, (1988 Apr 21) 332 (6166) 738-40.
Journal code: 0410462. ISSN: 0028-0836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198805
ED Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19880519

AB In humoral defence, pathogens are **cleared** by **antibodies** acting as adaptor molecules: they bind to **antigen** and trigger **clearance** mechanisms such as **phagocytosis**, **antibody**-dependent cell-mediated cytotoxicity and complement lysis. The first step in the complement cascade is the binding of Clq to the antibody. There are six heads on Clq, connected by collagen-like stems to a central stalk, and the isolated heads bind to the Fc portion of antibody rather weakly, with an affinity of 100 microM (ref. 3). Binding of antibody to multiple epitopes on an antigenic surface, aggregates the antibody and this facilitates the binding of several Clq heads, leading to an enhanced affinity of about 10 nM (ref. 1). Within the Fc portion of the antibody, Clq binds to the CH2 domain. The interaction is sensitive to ionic strength, and appears to be highly conserved throughout evolution as Clq reacts with IgG from different species (for example see ref. 8). By systematically altering surface residues in the mouse IgG2b isotype, we have localized the binding site for Clq to three side chains, Glu 318, Lys

320 and Lys 322. These residues are relatively conserved in other antibody isotypes, and a peptide mimic of this sequence is able to inhibit complement lysis. We propose that this sequence motif forms a common core in the interactions of IgG and Clq.

L2 ANSWER 20 OF 39 MEDLINE
AN 87188444 MEDLINE
DN 87188444 PubMed ID: 3568453
TI Studies in cobra venom factor treated rats of antibody coated erythrocyte clearance by the spleen: differential influence of red blood cell antigen number on the inhibitory effects of immune complexes on Fc dependent clearance.
AU Yousaf N; Howard J C; Williams B D
NC CA 34913 (NCI)
SO CLINICAL AND EXPERIMENTAL IMMUNOLOGY, (1986 Dec) 66 (3) 654-60.
Journal code: 0057202. ISSN: 0009-9104.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198706
ED Entered STN: 19900303
Last Updated on STN: 19970203
Entered Medline: 19870612
AB The splenic component of the mononuclear **phagocyte** system (MPS) was investigated in de complemented rats by determining the **clearance** from the blood of erythrocytes coated with a monoclonal **antibody** (R3/13). The infusion of immune complexes (IC), prepared at 10-fold **antigen** excess, at an appropriate time during the erythrocyte clearance produced a significant increase in the T_{1/2} of the antibody coated cells. Immune complexes formed with the F(ab')₂ fragment of the rabbit antibody did not have any significant effect. A positive correlation was seen between the dose of immune complex infused and the degree of inhibition of erythrocyte clearance. The influence of red cell antigen number on the behaviour of erythrocytes sensitized with R3/13 was studied by comparing the clearance of DA and (DA X PVG) F1 erythrocytes. F1 erythrocytes, with only half the number of specific antigens on their surface that bind R3/13 antibody were cleared much more slowly (82 +/- 2.6 min, mean +/- s.e.) by the spleen than the DA erythrocytes (44 +/- 1.5 min P less than 0.001). Both cell suspensions were equally susceptible to inhibition by soluble IC. These studies show that the number of specific antigens on the red cell surface influences the rate at which sensitized cells are removed by splenic macrophage Fc receptors but not their susceptibility to inhibition by IC. Our results draw attention to a major defect in the use of autologous erythrocytes coated with anti-rhesus (D) immunoglobulin to assess macrophage Fc receptor function in man.

L2 ANSWER 21 OF 39 MEDLINE
AN 87006836 MEDLINE
DN 87006836 PubMed ID: 3759129
TI Studies in the rat of antibody-coated and N-ethylmaleimide-treated erythrocyte clearance by the spleen. I. Effects of in vivo complement activation.
AU Yousaf N; Howard J C; Williams B D
NC CA 34913 (NCI)
SO IMMUNOLOGY, (1986 Sep) 59 (1) 75-9.
Journal code: 0374672. ISSN: 0019-2805.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198611

ED Entered STN: 19900302
Last Updated on STN: 19970203
Entered Medline: 19861112
AB The splenic component of the mononuclear **phagocyte** system (MPS) was investigated in the rat using N-ethylmaleimide-treated erythrocytes (NEM) and erythrocytes coated with a monoclonal IgG2b **antibody** (R3/13) directed against the rat RT1Aa major histocompatibility **antigen**. Both cell suspensions were removed by the spleen, and their **clearance** times were significantly longer in splenectomized animals. The mean clearance times for the NEM-treated cells in both normal and cobra venom-treated rats were similar (19.1 +/- 1.1 min and 19.0 +/- 1.0 min, respectively) but differences were seen between the clearance of R3/13 antibody-sensitized cells in these two groups (normal rats 38.3 +/- 2.8 min and CVF-treated rats 51.7 +/- 4.2 min, P less than 0.02). Different receptors were also involved in the removal of these cells; in normal animals recognition entailed interaction with complement receptors, whereas in CVF-treated animals this was implemented by Fc receptors. Complement activation prolonged the clearance rates of both R3/13 cells and NEM cells in normal animals, but the effect of complement activation on the clearance of NEM-treated cells was achieved via changes in splenic blood flow. When this was prevented from taking place no effect was seen on the clearance of NEM cells, although the clearance of R3/13 cells was inhibited by the complement fragments generated by complement activation.

L2 ANSWER 22 OF 39 MEDLINE
AN 86198516 MEDLINE
DN 86198516 PubMed ID: 3486243
TI Murine eosinophil differentiation factor. An eosinophil-specific colony-stimulating factor with activity for human cells.
AU Lopez A F; Begley C G; Williamson D J; Warren D J; Vadas M A; Sanderson C J
NC AI-21876 (NIAID)
SO JOURNAL OF EXPERIMENTAL MEDICINE, (1986 May 1) 163 (5) 1085-99.
Journal code: 2985109R. ISSN: 0022-1007.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198606
ED Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860606
AB A purified murine lymphokine, eosinophil differentiation factor (EDF), was found to be a selective stimulus for the clonal proliferation and differentiation of murine eosinophil progenitor cells, establishing it as the murine eosinophil colony-stimulating factor (Eo-CSF). EDF was also active on human eosinophil progenitors and mature blood eosinophils, but had no effect on neutrophil or macrophage precursor cells, nor on blood neutrophils. In culture of human bone marrow cells, EDF stimulated equal numbers and equal sizes of eosinophil colonies to develop when compared with human placental conditioned medium, a source of human CSFs, suggesting that all responsive progenitor cells were stimulated. Clone transfer experiments and the linear relationship between number of bone marrow cells plated and colonies produced confirmed that the action of EDF was directly on eosinophil progenitor cells. EDF increased the capacity of human blood eosinophils, but not neutrophils, to kill **antibody** -coated tumor cells and to **phagocytose** serum-opsonized yeast cells. This functional activation was associated with the enhanced expression of functional **antigens** (GFA-1, GFA-2, and the receptor for C3bi) on eosinophils. The possession by EDF (Eo-CSF) of all the properties expected of a human eosinophil CSF raises the possibility

that a human analog of this molecule exists, and is involved in the regulation of production and function of human eosinophils in vivo.

L2 ANSWER 23 OF 39 MEDLINE
AN 86170124 MEDLINE
DN 86170124 PubMed ID: 3514775
TI Type-specific antibody prevents platelet aggregation induced by group B streptococci type III.
AU Wood E G; Gray B M
NC 5 P01 HD17812 (NICHHD)
SO JOURNAL OF LABORATORY AND CLINICAL MEDICINE, (1986 Apr) 107 (4) 322-6.
Journal code: 0375375. ISSN: 0022-2143.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 198605
ED Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860501
AB Group B streptococci (GBS) type III organisms readily induced platelet aggregation and serotonin release in human platelet-rich plasma (PRP). In a system using a GBS/platelet ratio of 1.5, aggregation occurred after 2 to 9 minutes (maximum aggregation, 73% +/- 11%). Serotonin release began within the first minute, reaching 40% before aggregation was detected. Maximum release was 65% +/- 9%. The addition of type-specific rabbit antisera inhibited aggregation and release in a dose-dependent fashion, whereas rabbit antisera against GBS type II and a pneumococcus type 14 and 19 had no effect. To test the activity of different isotypes, monoclonal antibodies against the sialic acid determinant of the GBS type III **antigens** were used. IgG, IgM, and IgA **antibodies** were all effective in blocking aggregation and serotonin release. Although the significance of this phenomenon is not **clear**, it may represent a protective function of antibody that is not directly related to opsonization and **phagocytosis**.

L2 ANSWER 24 OF 39 MEDLINE
AN 85159059 MEDLINE
DN 85159059 PubMed ID: 3156932
TI Biochemical analysis and subcellular localization of a neutrophil-specific antigen, PMN-7, involved in the respiratory burst.
AU Melnick D A; Nauseef W M; Markowitz S D; Gardner J P; Malech H L
NC AI-06937 (NIAID)
AI-18166 (NIAID)
AI-19768 (NIAID)
+
SO JOURNAL OF IMMUNOLOGY, (1985 May) 134 (5) 3346-55.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 198505
ED Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19850523
AB The adherence of serum-opsonized yeast to neutrophils results in phagocytosis of these particulate stimuli and activation of the respiratory burst. Both events are mediated or modulated in part by the surface receptors for IgG and complement. The link between the binding of complex particulate stimuli to the cell surface, and the triggering of these neutrophil functions, is not completely understood. We have

previously described an anti-human neutrophil, murine monoclonal antibody PMN7C3, which specifically inhibits the respiratory burst of neutrophils stimulated with serum-opsonized yeast. In the present study, we show that the antigen recognized by PMN7C3 (PMN7 antigen) is present on a number of neutrophil proteins, including the recently described group of related leukocyte membrane glycoproteins CR3, LFA-1, and p150,95. The PMN-7 antigen differs from other antigens associated with the C3bi receptor complex (MAC 1, MO 1, OKM1, OKM10) in that it is present only on neutrophils among peripheral blood cells. Furthermore, the binding of PMN7C3 to the neutrophil surface inhibits the activation of the respiratory burst by serum **opsonized** zymosan without affecting **phagocytosis** of these particulate stimuli. The cross-linking of cell surface PMN7 **antigen** by multivalent **antibody** is associated with the capping and internalization of **antigen-antibody** complexes, and appears to be necessary for the expression of maximum inhibition of **opsonized** zymosan-triggered respiratory burst activity. PMN7C3 also binds to a group of granule-associated proteins biochemically distinct from CR3, LFA-1, and p150,95. These granule-associated proteins containing PMN7 antigen can be mobilized to the cell surface with secretion. PMN7 antigen-bearing proteins may play a role in modulating the activation of the respiratory burst associated with phagocytosis of serum-opsonized zymosan.

L2 ANSWER 25 OF 39 MEDLINE

AN 85078590 MEDLINE

DN 85078590 PubMed ID: 3917427

TI Effects of anthrax toxin components on human neutrophils.

AU O'Brien J; Friedlander A; Dreier T; Ezzell J; Leppla S

SO INFECTION AND IMMUNITY, (1985 Jan) 47 (1) 306-10.

Journal code: 0246127. ISSN: 0019-9567.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 198502

ED Entered STN: 19900320

Last Updated on STN: 19900320

Entered Medline: 19850215

AB The virulence of *Bacillus anthracis* has been attributed to a tripartite toxin composed of three proteins designated protective antigen, lethal factor, and edema factor. The effects of the toxin components on phagocytosis and chemiluminescence of human polymorphonuclear neutrophils were studied in vitro. Initially, it was determined that the avirulent Sterne strain of *B. anthracis* (radiation killed) required opsonization with either serum complement or **antibodies** against the Sterne cell wall to be **phagocytized**. **Phagocytosis** of the **opsonized** Sterne cells was not affected by the individual anthrax toxin components. However, a combination of protective **antigen** and edema factor inhibited Sterne cell **phagocytosis** and blocked both particulate and phorbol myristate acetate-induced polymorphonuclear neutrophil chemiluminescence. These polymorphonuclear neutrophil effects were reversible upon removal of the toxin components. The protective antigen-edema factor combination also increased intracellular cyclic AMP levels. These studies suggest that two of the protein components of anthrax toxin, edema factor and protective antigen, increase host susceptibility to infection by suppressing polymorphonuclear neutrophil function and impairing host resistance.

L2 ANSWER 26 OF 39 MEDLINE

AN 84069436 MEDLINE

DN 84069436 PubMed ID: 6417619

TI A pomona serogroup-specific, agglutinating antigen in *Leptospira*,

identified by monoclonal antibodies.

AU Adler B; Faine S
SO PATHOLOGY, (1983 Jul) 15 (3) 247-50.
Journal code: 0175411. ISSN: 0031-3025.
CY Australia
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198401
ED Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19840107

AB Monoclonal antibodies were produced by hybridoma cell lines derived by fusion of mouse NS-1 myeloma cells with splenocytes from mice immunized with *Leptospira interrogans* serovar pomona. One hybridoma (A3) produced an IgG2a antibody which agglutinated all leptospires of the Pomona serogroup but not leptospires representative of serovars of any other serogroup. The antibody precipitated in immunodiffusions with either alkali- or phenol-extracted lipopolysaccharide and also with the TM **antigen** of Yanagawa et al., indicating a common determinant on both **antigens**. A3 **antibody opsonized** viable leptospires for **phagocytosis** by mouse macrophages in vitro.

L2 ANSWER 27 OF 39 MEDLINE
AN 82184275 MEDLINE
DN 82184275 PubMed ID: 6280862
TI Characteristics of Hodgkin's disease-derived cell lines.
AU Diehl V; Kirchner H H; Burrichter H; Stein H; Fonatsch C; Gerdes J; Schaadt M; Heit W; Uchanska-Ziegler B; Ziegler A; Heintz F; Sueno K
SO CANCER TREATMENT REPORTS, (1982 Apr) 66 (4) 615-32.
Journal code: 7607107. ISSN: 0361-5960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198207
ED Entered STN: 19900317
Last Updated on STN: 19980206
Entered Medline: 19820708

AB In the last 3 years we were able to establish five long-term in vitro cell cultures from biopsy specimens taken preterminally from four patients with histologically proven Hodgkin's disease (nodular sclerosing type, clinical stage IVB). Four of the lines are continuously proliferating in vitro; one culture stopped growth for unknown reasons after 7 months. When culture conditions were modulated, the first culture, L 428, gave rise to two sublines: L 428 KS, after adaptation to calf serum, and L 428 KSA, permanently growing as an adherent monolayer line after treatment with a phorbol ester (12-O-tetradecanoylphorbol-13-acetate) for 3 weeks. Cell-marker analysis by conventional means (SIg, cIg, rosette formation, Epstein-barr virus reactivity, cytochemistry, **phagocytosis**, and lysozyme production) and with monoclonal **antibodies** directed against various human lymphoid, myeloid, and monocytoid **antigens** showed that the tested cell lines are **clearly** different from all hitherto described hematopoietic lines; they most likely represent a cell type resembling an early myeloid-monocytoid progenitor cell. Conditioned medium of the L 428 cells and its two sublines showed colony-stimulating factor activity and suppression of spontaneous cell-mediated cytotoxicity of L 428 KS and K 562 cells.

L2 ANSWER 28 OF 39 MEDLINE
AN 82051990 MEDLINE
DN 82051990 PubMed ID: 7028625

TI Human neutrophil swelling induced by immune complexes and aggregated IgG.
 AU Hurd E R; Hashimoto Y
 NC AM09989 (NIADDK)
 SO INFLAMMATION, (1981 Sep) 5 (3) 213-22.
 Journal code: 7600105. ISSN: 0360-3997.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 198201
 ED Entered STN: 19900316
 Last Updated on STN: 19970203
 Entered Medline: 19820128

AB Using a Coulter counter method, the effects of various types of IgG-dependent **phagocytic** stimuli on human neutrophil (PMN) swelling were determined. Human heat aggregated IgG, ovalbumin-antiovalbumin (OV-anti-OV) immune complexes, and **opsonized** latex particles all induced PMN swelling. The OV-anti-OV immune complexes were effective, whether prepared at **antigen-antibody** equivalence (insoluble) or at 4 or 9 times **antigen** excess (soluble). Swelling of PMN occurred at 37 degrees C, but not at 4 degrees C. Complement was not present in any of the experiments. In contrast to the above results, native IgG, OV-anti-OV F(ab')₂ immune complexes and unopsonized latex particles did not induce PMN swelling. These results suggest that the PMN swelling observed in this study is due to Fc-dependent, complement-independent membrane stimulation and/or phagocytosis.

L2 ANSWER 29 OF 39 MEDLINE
 AN 81238589 MEDLINE
 DN 81238589 PubMed ID: 7019072
 TI Artificial Salmonella vaccines: Salmonella typhimurium O-**antigen**-specific oligosaccharide-protein conjugates elicit **opsonizing antibodies** that enhance **phagocytosis**.

AU Jorbeck H J; Svenson S B; Lindberg A A
 SO INFECTION AND IMMUNITY, (1981 May) 32 (2) 497-502.
 Journal code: 0246127. ISSN: 0019-9567.

CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 198109
 ED Entered STN: 19900316
 Last Updated on STN: 19900316
 Entered Medline: 19810915

AB Outbred NMRI mice and rabbits were vaccinated with different artificial Salmonella typhimurium immunogens and the specificity and activity of elicited antibodies were studied in vivo and in vitro phagocytosis assays. The Salmonella immunogens used were: (i) the synthetic disaccharide, abequose (formula see text) D-mannose, representative of Salmonella O antigen 4, covalently linked to bovine serum albumin (BSA); (ii) the octa- and dodecasaccharides, (formula see text) covalently linked to BSA; and (iii) whole heat-killed Salmonella. Rabbit antibodies passively administered to mice significantly enhanced the clearance of intravenously injected S. typhimurium challenge bacteria from the bloodstream. The clearance rate and the titer of anti-O-antigen-specific antibodies correlated. The clearance rate of an S. thompson (O6,7) strain, which has a different O antigen, was the same irrespective of the rabbit serum given. NMRI mice actively immunized with the various oligosaccharide-BSA conjugates had a significantly increased clearance rate of S. typhimurium only. In the in vitro assay, mouse antioligosaccharide-BSA sera promoted phagocytosis of S. typhimurium, but

not S. thompson, when incubated with complement and mouse peritoneal exudate cells activated with Freund complete adjuvant.

- L2 ANSWER 30 OF 39 MEDLINE
AN 81095142 MEDLINE
DN 81095142 PubMed ID: 6450248
TI MLC-conditioned media stimulate an increase in Fc receptors on human macrophages.
AU Guyre P M; Crabtree G R; Bodwell J E; Munck A
NC AM 03535 (NIADDK)
CA 17323 (NCI)
CA23108 (NCI)
SO JOURNAL OF IMMUNOLOGY, (1981 Feb) 126 (2) 666-8.
Journal code: 2985117R. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 198103
ED Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19810324
AB Macrophage Fc receptors (FcR) are essential for **antibody**-dependent cellular cytotoxicity and for optimal **phagocytosis** of **opsonized** particulate **antigens**. Culture in the presence of conditioned medium from mixed leukocyte cultures (MLC-CM) resulted in a dose- and time-dependent increase (up to 10-fold) in FcR-dependent binding of 125I-labeled IgG1 to promyelocytic HL-60 cells, macrophage-like U-937 cells, and normal cultured human monocytes. FcR increase in HL-60 cells was blocked by cycloheximide (100 microM) and was accompanied by a slight decrease in binding affinity. Since cell volume did not change, the increase in FcR probably represents an increase in the surface density of FcR sites. MLC-CM prepared with or without serum were equally effective in augmenting FcR sites, whereas only serum-containing MLC-CM caused morphologic change of U-937 and HL-60 cells.
- L2 ANSWER 31 OF 39 MEDLINE
AN 80261393 MEDLINE
DN 80261393 PubMed ID: 6447414
TI [Significance of the immune complex reaction in internal medicine]. Bedeutung von Immunokomplexreaktionen in der Inneren Medizin.
AU Jager L; Herrmann D
SO ZEITSCHRIFT FUR DIE GESAMTE INNERE MEDIZIN UND IHRE GRENZGEBIETE, (1980 Jan 15) 35 (2) 49-57. Ref: 42
Journal code: 21730470R. ISSN: 0044-2542.
CY GERMANY, EAST: German Democratic Republic
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LA German
FS Priority Journals
EM 198010
ED Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19801021
AB A review is given concerning occurrence, identification, and pathogenetic effects of immune complexes. The formation of immune complexes by interaction of **antigen** with **antibody**, is a component of the normal immune response. In cases with inefficient **clearance** by the mononuclear **phagocyte** system only, pathological consequences will be expected, in particular by immune complexes formed with moderate excess of antigen. For the identification of immune complexes in biological fluids many methods have been developed. The

methods are based mostly on biological activities of immune complexes, e.g. interaction with complement or rheumatoid factor and reactivity with cellular receptors. These antigen-nonspecific methods do not allow to discriminate between true immune complexes and nonspecifically aggregated immunoglobulins. Circulating immune complexes become fixed to basement membranes of the body. They can produce an acute inflammatory reaction by activation of complement and inflammatory cells and also interfere with the immune response. Circulating immune complexes have been detected in some human diseases, e.g. autoimmune diseases (LEV, RA), infectious diseases, malignancies, serum sickness syndrome, immune-complex glomerulonephritis, and after transplantation. The possibilities for management of immune complex diseases are discussed.

L2 ANSWER 32 OF 39 MEDLINE
 AN 80048686 MEDLINE
 DN 80048686 PubMed ID: 91583
 TI Immunological specificity of natural opsonins and their role in the cross-reactivity between Staphylococcus aureus Mardi and Escherichia coli 101.
 AU Young D A; Dobson P; Karakawa W W
 SO INFECTION AND IMMUNITY, (1979 Sep) 25 (3) 954-9.
 Journal code: 0246127. ISSN: 0019-9567.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 198001
 ED Entered STN: 19900315
 Last Updated on STN: 19900315
 Entered Medline: 19800128
 AB The immunochemical specificity of the observed cross-reactivity between Escherichia coli strain 101 and Staphylococcus aureus strain Mardi was examined. The cross-reactivity was shown to be dependent upon mucopeptide antibodies which are present in normal and immune sera. Although both organisms contained surface **antigens** with immunodominant glucuronic acid residues, in vitro **phagocytosis** studies indicated that **antibodies** directed against these **antigens** were not significantly involved in the **opsonization** process. Rather, **antibodies** with mucopeptide specificity were shown to be involved in the in vitro **phagocytosis** of these organisms by polymorphonuclear leukocytes. The mucopeptide **antibodies**, which were found in both nonimmune and immune sera, were shown to be effective in **opsonizing** both the S. aureus strain and the E. coli strain. The ubiquitous distribution of E. coli strains containing mucopeptide **antigens** common to most bacteria suggests that these organisms may be responsible for the wide prevalence of natural staphylococcal opsonins with mucopeptide specificity in normal sera.

L2 ANSWER 33 OF 39 MEDLINE
 AN 79216740 MEDLINE
 DN 79216740 PubMed ID: 457853
 TI Immunochemical study of diverse surface antigens of a Staphylococcus aureus isolate from an osteomyelitis patient and their role in in vitro phagocytosis.
 AU Karakawa W W; Young D A
 SO JOURNAL OF CLINICAL MICROBIOLOGY, (1979 Mar) 9 (3) 399-408.
 Journal code: 7505564. ISSN: 0095-1137.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals

EM 197909
ED Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19790925

AB The cellular antigens of a strain of *Staphylococcus aureus*, isolated from a bone fragment from osteomyelitis, were analyzed immunochemically and by interaction with human phagocytic cells. When this strain was allowed to interact with human polymorphonuclear cells in the presence of antiserum, the strain was shown to have specific antiphagocytic antigens. An acidic polysaccharide consisting of galactose and glucuronic acid was isolated from the cell surface of the organism, and in vitro **opsonization** tests indicated that this acidic **antigen** impeded in vitro **phagocytosis** by human polymorphonuclear cells. It was also observed that **antibodies** directed against the mucopeptide constituents of homologous and heterologous bacterial cell walls were effective in promoting the in vitro **opsonization** of the organism. In the presence of antimucopeptide serum and human polymorphonuclear cells, a variant strain was isolated from the wild type, and immunochemical analysis indicated that this strain consisted of galactose and immunodominant amino-galacturonic acid residues. In vitro phagocytosis studies employing this variant strain indicated that the homologous human convalescent serum contained higher levels of opsonins against the variant strain than the original isolate, the wild type. This observation is discussed.

L2 ANSWER 34 OF 39 MEDLINE
AN 79195743 MEDLINE
DN 79195743 PubMed ID: 448288
TI Immune phagocytosis in murine malaria.
AU Shear H L; Nussenzweig R S; Bianco C
SO JOURNAL OF EXPERIMENTAL MEDICINE, (1979 Jun 1) 149 (6) 1288-98.
Journal code: 2985109R. ISSN: 0022-1007.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197909
ED Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19790901

AB Spleen macrophages from *Plasmodium berghei*-infected mice are more efficient in the ingestion of parasitized reticulocytes than spleen macrophages obtained from normal animals. Other indications of spleen macrophage activation detected during malarial infection are enhanced macrophage spreading and increased **phagocytosis** of **opsonized** and nonopsonized sheep erythrocytes (E). Peritoneal macrophages are not activated to a significant degree. The appearance of **antibodies** directed against Forssman **antigen**, but not to other erythrocyte **antigens**, is also a feature of this infection and explains the ingestion of unsensitized E by spleen macrophages of the diseased animals. The recognition and ingestion of parasitized reticulocytes by infected mice is mediated by cold-agglutinin type immunoglobulins that appear during *P. berghei* infection and can be blocked by the Fc-binding protein A from *Staphylococcus aureus*. In advanced stages of the disease, the serum of infected animals inhibits phagocytosis, probably because of the high level of circulating immune complexes. Thus, the clearance of malaria parasites is regulated by several elements of the immune system, in addition to levels of specific antimerozoite antibodies, including the amount of antibodies bound to reticulocytes, the presence of circulating immune complexes, and the degree of macrophage stimulation.

L2 ANSWER 35 OF 39 MEDLINE

AN 79173444 MEDLINE
 DN 79173444 PubMed ID: 220283
 TI In vitro suppression of serum elastase-inhibitory capacity by reactive oxygen species generated by phagocytosing polymorphonuclear leukocytes.
 AU Carp H; Janoff A
 SO JOURNAL OF CLINICAL INVESTIGATION, (1979 Apr) 63 (4) 793-7.
 Journal code: 7802877. ISSN: 0021-9738.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Abridged Index Medicus Journals; Priority Journals
 EM 197907
 ED Entered STN: 19900315
 Last Updated on STN: 20000303
 Entered Medline: 19790725
 AB Human polymorphonuclear leukocytes (PMN) **phagocytosing opsonized antigen-antibody** complexes, produce dialyzable species of activated oxygen which are capable of partially suppressing the elastase-inhibiting capacity (EIC) of whole human serum or purified human alpha1-proteinase inhibitor. Serum EIC was partially protially protected by superoxide dismutase, catalase, or mannitol, suggesting that hydroxyl radical, formed by interaction of superoxide radical and hydrogen peroxide, might be responsible for this effect. NaN3 also partly protected EIC, implicating myeloperoxidase-mediated reactions as well. An artificial superoxide rradical-generating system, involving xanthine and xanthine-oxidase, could be substituted for phagocytosing PMN with resultant EIC suppression. These results are consistent with previous demonstrations of the release of potent oxidants by stimulated PMN, as well as earlier studies from our laboratory showing sensitivity of alpha1-proteinase inhibitor to inactivation by oxidants. Oxidative inactivation of proteinase inhibitors in the microenvironment of PMN accumulating at sites of inflammation may allow proteases released from these cells to more readily damage adjacent connective tissue structures.

L2 ANSWER 36 OF 39 MEDLINE
 AN 79049305 MEDLINE
 DN 79049305 PubMed ID: 309494
 TI Role of immunity in the clearance of bacteremia due to Haemophilus influenzae.
 AU Weller P F; Smith A L; Smith D H; Anderson P
 SO JOURNAL OF INFECTIOUS DISEASES, (1978 Oct) 138 (4) 427-36.
 Journal code: 0413675. ISSN: 0022-1899.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Abridged Index Medicus Journals; Priority Journals
 EM 197901
 ED Entered STN: 19900314
 Last Updated on STN: 19900314
 Entered Medline: 19790115
 AB The role of antibodies to capsular and somatic antigens in the clearance of Haemophilus influenzae was investigated by active and passive immunization. The clearance index (k) and the proportion of strain b organisms cleared 30 min after intravenous administration (deltaY30) were greater in eight-week-old actively immunized rats (k = 0.693, deltaY30 = 4.07) than in nonimmune animals (k = 0.075, deltaY30 = 0.95) (P less than 0.025 for all); however, clearance correlated imprecisely with titers of bactericidal or anticapsular antibody. In three-week-old rats, intranasal immunization with strain b or U significantly increased (P less than 0.005) the rate of clearance of strains b and U. Passive immunization with antibodies to somatic or capsular antigens significantly increased the rate of clearance (P less than 0.001) and the proportion of bacteria

cleared (P less than 0.05) with all test strains. The increased clearance associated with passive immunization correlated with increased splenic uptake of 32P-labeled H. influenzae ($r = 0.83$, P less than 0.025). Analysis of the disappearance of viable organisms and bacterial 32P suggested that bacteriolysis of H. influenzae did not occur during **clearance** of the bacteremia. Either **antibody** to capsular **antigen** or **antibody** to somatic **antigen**, administered or evoked in rats, accelerates intravenous **clearance** of H. influenzae by promotion of reticuloendothelial **phagocytosis**

L2 ANSWER 37 OF 39 MEDLINE
 AN 79018611 MEDLINE
 DN 79018611 PubMed ID: 29470
 TI Immunological properties of the lipopolysaccharide-protein complex of Coxiella burnetii.
 AU Kazar J; Schramek S; Brezina R
 SO ACTA VIROLOGICA, (1978 Jul) 22 (4) 309-15.
 Journal code: 0370401. ISSN: 0001-723X.
 CY Czechoslovakia
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 197811
 ED Entered STN: 19900314
 Last Updated on STN: 19950206
 Entered Medline: 19781129
 AB Purified lipopolysaccharide-protein complex (LPS-PC) extracted by trichloroacetic acid from phase I Coxiella burnetii organisms induced in mice and rabbits fair levels of **antibodies** directed to **antigen** 1 and **antigen** 2, as detected by complement-fixation (CF), microagglutination (MA), **opsonization-phagocytosis** (OP) and serum protection (SP) tests. In guinea pigs only very low levels of MA **antibodies** against **antigen** 2 were demonstrated. In rabbit serum, MA antibodies directed to **antigen** 2 were found exclusively in the IgM fraction after the primary immunizing dose; the second dose was followed by gradual shift of MA antibodies to the IgG class. Two immunizing doses of the LPS-PC were more effective when testing antibody response in mice or protection of mice and guinea pigs against phase I virulent challenge.

L2 ANSWER 38 OF 39 MEDLINE
 AN 76203262 MEDLINE
 DN 76203262 PubMed ID: 1274982
 TI Selective immunoglobulin M (IgM) deficiency in two immunodeficient adults with recurrent staphylococcal pyoderma.
 AU Yocum M W; Strong D M; Chusid M J; Lakin J D
 SO AMERICAN JOURNAL OF MEDICINE, (1976 Apr) 60 (4) 486-94.
 Journal code: 0267200. ISSN: 0002-9343.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Abridged Index Medicus Journals; Priority Journals
 EM 197608
 ED Entered STN: 19900313
 Last Updated on STN: 19900313
 Entered Medline: 19760802
 AB Two adult men with recurrent pyoderma due to Staphylococcus aureus and a selective deficiency of immunoglobulin M (IgM) **antibody** synthesis are described. An analysis of each patient's polymorphonuclear leukocyte chemotaxis, **phagocytosis** and killing of Staph. aureus, serum **opsonization** of Staph. aureus, and serum and

lymphocyte-mediated responses to **antigenic** stimulation was performed. Family studies revealed a possible autosomal dominant inheritance pattern with heterogenetic expression of various dysgammaglobulinemic states in each patient's first degree relatives. In vivo studies of delayed hypersensitivity and in vitro studies of polymorphonuclear leukocyte and lymphocyte function were normal. A defect in IgM, but not in IgG (immunoglobulin G), antibody synthesis to a number of antigens, and a mild decrease in serum opsonic activity to Staph. aureus correctable by heat inactivated normal human serum were found in each patient. In these patients, the recurrent staphylococcal pyoderma prompted an investigation of host defense mechanisms and revealed low to absent IgM levels and a defect in IgM antibody synthesis.

L2 ANSWER 39 OF 39 MEDLINE
 AN 76170837 MEDLINE
 DN 76170837 PubMed ID: 772153
 TI Immunity to the group B streptococci: interaction of serum and macrophages with types Ia, Ib, and Ic.
 AU Anthony B F
 SO JOURNAL OF EXPERIMENTAL MEDICINE, (1976 May 1) 143 (5) 1186-98.
 Journal code: 2985109R. ISSN: 0022-1007.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Abridged Index Medicus Journals; Priority Journals
 EM 197607
 ED Entered STN: 19900313
 Last Updated on STN: 19900313
 Entered Medline: 19760706
 AB The opsonization and phagocytosis of group B streptococci of types Ia, Ib, and Ic were studied in vitro by measuring the uptake of radioactivity by coverslip cultures of rabbit alveolar macrophages during incubation with radiolabeled, nonviable bacteria which had been exposed to rabbit serum. The uptake of counts per minute was quantitative, reproducible, and reversibly inhibited by cold, indicating that it was largely a measurement of phagocytic ingestion rather than of attachment of bacteria-immunoglobulin complexes to macrophage membranes. Moreover, suspended macrophages killed approximately 90% of viable streptococci in the presence of specific antiserum. The opsonic activity of immune serum was heat stable, and phagocytosis of streptococci was insignificant after incubation with normal serum and antiserum to some heterologous group B streptococci. By absorption studies, it was possible to identify the effect of **antibodies** to specific bacterial **antigens**.
Phagocytosis of streptococci containing the corresponding **antigens** was maximal after **opsonization** with homologous or heterologous sera containing **antibody** to IaCHO, IbCHO, or Ibc protein. **Phagocytosis** of all three serotypes was intermediate when **opsonization** could be attributed to anti-IabcCHO. The **opsonization** of a specific group B streptococcus is complex and may involve two or more **antigen-antibody** systems.

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L8: Entry 3 of 3

File: USPT

Aug 3, 1993

DOCUMENT-IDENTIFIER: US 5233024 A

TITLE: Anti-idiotypic monoclonal antibodies for mucoid *Pseudomonas aeruginosa*, their preparation and useUS PATENT NO. (1):
5233024Abstract Text (1):

An anti-idiotypic monoclonal antibody, which induces production of mucoid exopolysaccharide-specific antibodies which are opsonic for mucoid *Pseudomonas aeruginosa*. The anti-idiotypic monoclonal antibody is produced by a cell line designated C9F5 and having ATCC accession No. HB10715. The anti-idiotypic monoclonal antibody is useful as a vaccine and for diagnostic purposes.

Brief Summary Text (2):

The present invention relates generally to infectious diseases and antibodies, and, more particularly, to a new hybridoma cell line for production of anti-idiotypic monoclonal antibodies directed to an opsonic monoclonal antibody specific to mucoid exopolysaccharide of *Pseudomonas aeruginosa*.

Brief Summary Text (4):

Mucoid strains of *Pseudomonas aeruginosa* are the primary pulmonary pathogen for cystic fibrosis (CF) patients. Acquisition of this organism in the lungs is invariably associated with clinical decline, and there is a strong association between expression of the mucoid phenotype and growth in the lungs of CF patients. Mucoid exopolysaccharide (MEP), the primary constituent of the extracellular slime coat of mucoid strains, appears to be an important antigen in the pathophysiology of *P. aeruginosa* infection of CF patients. MEP expression promotes adherence of mucoid *P. aeruginosa* to tracheal cells and to respiratory mucins and antibodies to MEP are important to host defenses against the organism. According to earlier work (see Pier, G. B., et al., N. Engl. J. Med. 1987; 317: 793-798) induction of opsonic antibodies to MEP will probably be an important property of vaccines considered as candidates for prevention of mucoid *P. aeruginosa* infection in CF patients. Most CF patients respond to infection with high titers of nonopsonic antibody to MEP, and these antibodies fail to prevent progression of the infection. However, a small number of older (>12 years) CF patients have opsonic antibodies to MEP, are not infected with *P. aeruginosa*, and have an overall better clinical status. Furthermore, opsonic antibodies protect experimental animals from chronic mucoid *P. aeruginosa* lung infections. Pier, G. B., et al., Science 1990; 249: 537-540, the contents of which are incorporated herein by reference.

Brief Summary Text (5):

Recently, it has been observed that the heteropolymeric nature of MEP results in the presence of both common and type-specific epitopes. In addition, the common epitopes are further divided into those that bind opsonizing antibodies and those that bind nonopsonizing antibody. Most naturally occurring antibodies to MEP function poorly in in vitro opsonophagocytic assays with complement, and are unable to protect animals following intratracheal challenge with bacteria encased in agar beads. By contrast, antibodies that are highly opsonic protect animals against infection and are found among some older CF patients who are not colonized with *P. aeruginosa*. Opsonizing antibodies to MEP are usually not found in younger noncolonized or chronically colonized CF patients. These findings have suggested a protective effect for the opsonizing antibodies. It is believed that opsonizing antibodies to the MEP antigen will generally protect animals, including humans, livestock, and mammals generally, from infection. MEP has become a promising vaccine candidate for the prevention of

infection with *P. aeruginosa* in CF patients. Unfortunately, MEP appears to be poorly immunogenic in humans for the induction of opsonic antibodies. MEP does not readily elicit opsonic antibodies either during chronic infection or after vaccination.

Brief Summary Text (6):

Alternative strategies to immunization with purified bacterial polysaccharides include the use of polysaccharide-protein conjugates and anti-idiotypic antibodies as substitute antigens. Anti-idiotypes directed to the antigen binding site of other antibodies may function as "internal images" of antigen and induce antigen-specific antibodies in animals without exposure to nominal antigen. See Stein, K. E., Bona, C. A. ed. pp. 2-11, CRC Press, 1988, which is incorporated herein by reference. In addition, since anti-idiotypes are proteins, they are potentially more immunogenic than polysaccharides, particularly in young children. Anti-idiotypes have been used to induce antibodies to capsular polysaccharides and thus provide immunity to several bacterial pathogens including *Escherichia coli* (Stein, K. E., and Soderstrom, T., J. Exp. Med. 1984; 160:1001, incorporated herein by reference) and group C *Neisseria meningitidis* (Westerink, M. A. J., et al., Infect. Immun., 1988; 56:1120-1127, incorporated herein by reference). An anti-idiotypic that is a functional mimic of the immunotype-1 (IT 1) LPS O side chain of *P. aeruginosa* is capable of generating protective antibodies in syngeneic mice (Schreiber, J. R., et al., J. Immunol., 1990; 144:1023-1029, incorporated herein by reference). The present invention characterizes another monoclonal anti-idiotypic directed to an opsonic murine monoclonal antibody to MEP.

Brief Summary Text (8):

The present invention provides a monoclonal anti-idiotypic antibody which induces protective immunity in animal models. All experimental data to date, including animal testing, indicates that it can be used as a vaccine to generate protective immunity and prevent infections in humans, livestock, animals, and mammals in general. It would be of particular use to humans suffering from cystic fibrosis, burn patients, and those suffering from cancer. It can be used as the base for immunodiagnostics, including immunodiagnostic methods, testing, and reagents, to detect antibodies in humans and other animals and mammals. The monoclonal antibody of the present invention performs or acts as a molecular mimic of the MEP antigen of *P. aeruginosa*.

Brief Summary Text (9):

Initially, a murine monoclonal anti-idiotypic antibody directed to an opsonic monoclonal antibody (MAB) specific to MEP was produced. This anti-idiotypic MAB bound to F(ab')₂ fragments of the opsonic MAB, and blocked binding of the opsonic MAB to MEP. The murine anti-idiotypic also bound to human opsonic antibodies from individuals immunized with MEP vaccine, providing additional evidence for the antigen-binding-site specificity of the anti-idiotypic, as well as the presence of cross-reactive idiotopes on human and murine opsonic antibodies to MEP. In addition, the anti-idiotypic induced MEP-specific (primarily of the IgM class) antibodies in syngeneic mice. In allogeneic mice, both IgM and IgG_{sub.1} antibodies to the anti-idiotypic were elicited; these antibodies fixed complement onto the bacterial surface and opsonized mucoid *P. aeruginosa* for uptake and phagocytic killing by human peripheral blood leukocytes. These studies demonstrate the utility of anti-idiotypic MAB for generating protective immunity against mucoid strains of *P. aeruginosa*.

Detailed Description Text (5):

Mucoid *P. aeruginosa* strain 2192, a clinical isolate from the sputum of a patient with CF, was used as the target organism in phagocytic assays and complement deposition assays and as a source of purified MEP. MEP was purified as previously described. Garner, C. V., et al., Infect. Immun. 1990; 58:1835-1842, incorporated herein by reference. In the Example herein, smaller sized polymers of MEP (K_{sub.av} on a Sepharose CL4B column of <0.3) were used in ELISA and moderate sized polymers (K_{sub.av} = 0.1-0.3) were used for immunizing animals.

Detailed Description Text (7):

Production of opsonic MAb1 directed to *P. aeruginosa* MEP was accomplished by standard techniques. Spleens from mice immunized with live mucoid *P. aeruginosa* organisms were fused with myeloma cell line SP2/O-Ag14 and wells with growth following hypoxanthine-aminopterin selection screened against purified MEP in an ELISA, as previously described. (Bryan, L. E., et al., J. Clin. Microbiol. 1983; 18:276-282 and Pier, G. B., et al., J. Clin. Microbiol. 1986; 24:189-196, both incorporated herein by reference). Cells in wells positive for antibody to MEP were cloned twice by limiting dilution, and the antibodies were tested for opsonic activity in a phagocytic assay

described below. Two positive clones secreting IgG.sub.2b antibodies were used in the experiments reported here.

Detailed Description Text (9):

The anti-idiotypes were made by immunizing 8-wk.-old BALB/cByJ mice subcutaneously (sc) with 10 ug of the opsonic anti-MEP MAb (MAb1) in complete Freund's adjuvant and then with 10 ug MAb1 in incomplete Freund's adjuvant twice per week for 4 weeks. Anti-idiotypic-induced seroconversion to MAb1 was documented by ELISA screening (see below) using F(ab').sub.2 fragments from MAb1. The spleen of one mouse was then obtained 72 hours after the final immunization and used for production of hybridomas. Hybridomas were produced via fusion with the SP2/O mouse myeloma cell line, screened by ELISA for production of anti-idiotypic antibodies, and then cloned by repeated limiting dilution. Anti-idiotypic antibodies were purified from ascites fluid of pristane-primed BALB/cByJ mice by passage over a protein A-Sepharose column (Pharmacia Fine Chemicals, Piscataway, N.J.), as described in Schreiber, J. R., et al., J. Immunol. 1990; 144:1023-1029, incorporated herein by reference. One clone, C9F5, was found to be stable and to produce large quantities of antibody of the IgG2a isotype; it was used for further experiments.

Detailed Description Text (10):

D. Induction of MEP-specific antibodies in mice by immunization with anti-idiotypic MAB

Detailed Description Text (11):

To determine whether MEP-specific antibodies could be induced in 8-wk.-old syngeneic BALB/cByJ mice, the animals were immunized intraperitoneally (ip) or sc with 20- ug doses of anti-idiotypic MAB C9F5 without adjuvants. The immunization schedule was either two doses separated by three weeks or one dose per week for 4 weeks. In order to determine whether antibodies to MEP could be induced in allogeneic C3H/HeN mice, these animals were immunized with varying doses (0.5-50 ug) of the C9F5 anti-idiotypic weekly for 4 weeks. Serum was obtained weekly from all mice by tail vein bleeding, pooled and screened for binding to MEP by ELISA. Control animals received an irrelevant MAB of the same isotype by the same method of administration as the anti-idiotypic MAB C9F5 (IgG2a anti-Sendai virus kindly supplied by Drs. John Nedrud and Mary Mazanec, Case Western Reserve University School of Medicine, Cleveland, Ohio). For purposes of comparison, some animals received 1-ug doses of purified MEP antigen.

Detailed Description Text (12):

E. ELISA for detection of antibodies

Detailed Description Text (13):

Supernates from clones or mouse sera were screened for anti-idiotypic antibodies in an ELISA using plates sensitized with F(ab').sub.2 fragments from the anti-MEP MAB1. The F(ab').sub.2 fragments were prepared as described in Schreiber, J. R., et al., J. Immunol. 1990; 144:1023-1029, and Lamoyi, E., et al., J. Immunol. Methods 1983; 56:235-243, incorporated herein by reference. Each F(ab').sub.2 preparation was screened for purity by an ELISA with goat anti-mouse IgG Fc-specific antibodies conjugated to alkaline phosphatase (AP), and by SDS-PAGE. ELISA plates were coated with 100 ul/well of F(ab').sub.2 fragments (2.5 ug/ml concentration), then blocked with PBS containing 1% BSA for 60 minutes. After washing, sera or supernates were added, followed by goat anti-mouse IgG Fc fragment-specific or anti-mouse IgM, u-chain specific AP conjugates (Cappel Antibodies, Westchester, Pa.). The ELISA was developed with phosphatase substrate (1 mg/ml in diethanolamine, 0.5 mM MgCl.sub.2, 0.02% sodium azide, pH 9.8 [DEA buffer], Sigma, St. Louis, Mo.). Plates were read at 410 nm with a Titertek Multiscan ELISA plate reader (Flow Laboratories, Mclean, Va.).

Detailed Description Text (14):

Sera or tissue culture supernates were screened for MEP-specific antibodies by coating ELISA plates with 100 ul/well of 2.5 ug tyraminated MEP/ml. Tyramination was performed as described in Schreiber, J. R., et al., J. Immunol. 1990; 144:1023-1029, with cyanogen bromide coupling and was found to enhance binding of MEP to the ELISA plates. After blocking with 1% BSA in PBS, serum or tissue culture supernate was added. IgG, IgM, or IgA antibodies to MEP were detected with AP-conjugated, class- and subclass-specific goat anti-mouse Ig (Southern Biotech, Birmingham, Al.). Plates were developed as described above. Specificity of antibody to MEP in sera or tissue culture supernates was confirmed by preincubating these specimens with MEP (100 ug/ml) and observing inhibition of antibody binding to solid phase MEP in the ELISA.

Detailed Description Text (18):

G. Detection of binding of anti-idiotypic MAb to human antibodies to MEP

Detailed Description Text (19):

Adult volunteers with pre-existing nonopsonic antibody to MEP were immunized with 100 ug of an MEP vaccine as described in Garner, C. V., et al., Clin. Res. 1988; 36:465A. The MEP-specific antibodies from pre- and post-vaccination sera of four individuals who responded with opsonic antibody to MEP were then isolated by affinity chromatography, using a column of epoxy-activated Sepharose coupled to purified MEP. From these preparations, the IgG fraction was obtained by chromatography on protein A Sepharose. Fab fragments were prepared from these antibodies by incubating the affinity-purified material with papain immobilized on Sepharose. The digests were again passed over a protein A column to remove undigested antibody and Fc fragments. Characterization of the preparations by SDS-PAGE revealed no intact heavy chains. ELISA plates were coated with 1 ug of affinity-purified human antibody or Fab fragments in carbonate-bicarbonate buffer, pH 9.6. After sensitization for 2 hours at 37.degree. C., the plates were washed and blocked with 5% skim milk in PBS, and the protein A purified anti-idiotypic MAb C9F5 was added. After another 2 hours at 37.degree. C., the plates were washed, and AP-conjugated goat anti-mouse IgG diluted in PBS with 0.05% tween, 5% skim milk, and 2% normal human serum was added. After 2 more hours at 37.degree. C., the plates were washed, and paranitrophenol phosphate in 0.1M carbonate buffer containing 100 mg/L of MgCl.sub.2 was added. Absorbance was read after 60 minutes at 405 nm.

Detailed Description Text (20):

H. Measurement of opsonic activity of anti-idiotypic-induced MEP-specified antibodies

Detailed Description Text (24):

Several immunization regimens were employed to induce circulating anti-idiotypic antibodies in BALB/cByJ mice. Twice weekly administration of 10 ug of anti-MEP MAb1 antibodies in BALB/cByJ mice. Twice weekly administration of 10 ug of anti-MEP MAb1 initially with complete Freund's adjuvant and then with incomplete Freund's adjuvant, consistently produced seroconversion by week 4 as determined in the ELISA with F(ab').sub.2 of anti-MEP MAb1 as coating antigen. Seven hybridomas from fusion experiments were found to produce anti-idiotypic antibodies. One stable hybridoma was cloned repeatedly by limiting dilution and was chosen for further experiments (C9F5, IgG2a subclass). The specificity of antibodies from this clone was measured by coating microtiter wells with F(ab').sub.2 fragments from the anti-MEP MAb1, adding the C9F5 antibody, and then using an AP-conjugated goat anti-mouse IgG Fc-specific antibody as described in Materials and Methods. The C9F5 anti-idiotypic bound to F(ab').sub.2 of anti-MEP MAb1 in a dose-dependent fashion. The anti-idiotypic did not bind to F(ab').sub.2 fragments from other murine MABs, and the control MAB of the same isotype as the anti-idiotypic did not bind to the anti-MEP MAb1 F(ab').sub.2 fragments.

Detailed Description Text (26):

To determine whether the anti-idiotypic structurally resembled MEP, microtiter plates were coated with tyraminated MEP, and anti-MEP MAb1 that had been incubated with various quantities of anti-idiotypic was added. Anti-idiotypic inhibited binding of the anti-MEP MAb1 to MEP in a dose-dependent manner. By contrast, a control antibody of the same isotype and quantity as the anti-idiotypic did not inhibit binding of anti-MEP MAb1 to antigen in a dose-dependent fashion. These findings suggested that the C9F5 anti-idiotypic MAB functioned in vitro as a molecular mimic of MEP.

Detailed Description Text (27):

C. Binding of the anti-idiotypic MAB to human antibodies to MEP

Detailed Description Text (28):

Immunization of humans with MEP resulted in production of opsonic antibody in about 20% of the vaccinees, Garner, C. V. and Pier, G. B., Clin. Res., 1988; 36:465A. Whether anti-idiotypic C9F5 bound to intact, affinity-purified antibodies to MEP and their Fab fragments coated onto ELISA plates was tested, using the preimmunization sera from four of these individuals as a source of nonopsonic antibody and the postimmunization sera as a source of opsonic antibody. Intact opsonic antibodies from all four individuals bound the anti-idiotypic, as did Fab preparations from three of four individuals. None of the nonopsonic preparations bound to the anti-idiotypic. Further studies showed that, in the presence of purified MEP (100 ug/ml), the anti-idiotypic MAB did not bind to the opsonic antibody preparations.

Detailed Description Text (29):

D. Induction of anti-MEP antibodies in syngeneic BALB/cByJ mice with anti-idiotypic MAB

Detailed Description Text (30):

Since the C9F5 anti-idiotypic bound to F(ab')₂ of murine anti-MEP MAb1 as well as to human opsonic antibodies and Fab fragments in a manner suggestive of molecular mimicry of MEP, whether administration of anti-idiotypic to syngeneic mice would elicit antibodies to MEP was investigated. Seven BALB/cByJ mice were injected with 20 ug of anti-idiotypic MAb or control antibody in two doses given 3 weeks apart or in one dose per week for 4 weeks. Each mouse was bled weekly to determine the presence of antibodies to MEP as measured by binding to tyraminated-MEP-coated ELISA plates. Previous experiments had determined that 20 ug of the anti-idiotypic MAb yielded the highest antibody response to MEP. Administration of the anti-idiotypic MAb by these two schedules yielded anti-MEP antibody of the IgM class by day 7 post-immunization. Higher titers were obtained with repetitive doses, but the antibody remained solely of the IgM class. Control animals immunized with four weekly 20-ug doses of an IgG.sub.2a antibody against Sendai virus made no antibody response to MEP. BALB/cByJ mice given 10 ug of purified MEP antigen produced a detectable antibody response to MEP within 7 days of immunization, as has been previously described in Garner, C. V., et al., Infect. Immun. 1990; 58:1835-1842. Both IgM and IgG3 antibodies were detected, a finding consistent with previous reports of the isotypically restricted murine antibody response to purified polysaccharides. (Perlmutter, R., et al., J. Immunol. 1978; 121:566-572)

Detailed Description Text (31):

E. Induction of antibodies to MEP in allogeneic C3H mice by anti-idiotypic MAb

Detailed Description Text (32):

Anti-idiotypic MAb C9F5 administered in doses of 0.5-50 ug to allogeneic C3H/HeN mice resulted in production of antibodies to MEP. As with the BALB/cByJ mice, anti-idiotypic MAb given once per week for 4 weeks induced detectable IgM antibodies at most of the doses 7 days after the initial immunization. These IgM antibodies tended to decline after this initial period. In contrast to the results in syngeneic BALB/cByJ mice, IgG.sub.1 antibodies to MEP were elicited in the C3H/HeN mice by week 3 when doses of the anti-idiotypic MAb of .gtoreq.10 ug were used, and by week 4 when doses of 0.5 and 1 ug were given. IgG.sub.1 is the murine isotype commonly associated with immune responses to protein antigens. Anti-idiotypic MAb C9F5 is a protein.

Detailed Description Text (33):

F. Opsonic ability of the anti-idiotypic-induced antibodies to MEP

Detailed Description Text (34):

Next, whether the antibodies induced by the anti-idiotypic MAb could opsonize mucoid P. aeruginosa for killing by human leukocytes was addressed. C3H/HeN mice were immunized once per week for 4 weeks with 0.5 to 50 ug of the anti-idiotypic MAb C9F5. Killing of mucoid P. aeruginosa strain 2192 was assessed after 90 minutes in an opsonophagocytic assay by a 1:8 serum dilution. Serum obtained 3 to 4 weeks after immunization with the anti-idiotypic MAb led to peripheral blood leukocyte killing of >80% of mucoid P. aeruginosa. Development of opsonizing antibody was coincident with the appearance of IgG.sub.1. Serum from control mice immunized with 1 ug of MEP once per week for 4 weeks also produced opsonophagocytic killing 3 weeks after the initial dose. No phagocytic killing was observed if complement, antibody or phagocytic cells were omitted from the assay. Serum obtained from BALB/cByJ mice immunized with anti-idiotypic was poorly opsonic, perhaps due to restriction of the response to the IgM isotype.

Detailed Description Text (35):

G. Fixation of complement to the surface of mucoid P. aeruginosa by the anti-idiotypic-induced, MEP-specific antibodies

Detailed Description Text (36):

Whether the anti-idiotypic-induced MEP-specific antibodies could fix complement to the bacterial surface was addressed. Deposition of the third component of complement onto antibody-opsonized mucoid P. aeruginosa was studied. Bacteria were incubated for 5 minutes with sera from C3H/HeN mice, .sup.3 H-labeled C3, and 0.5% intact human serum as a complement source. C3 binding was detected by scintillation counting following washing in 1% SDS in PBS. Sera from C3H mice immunized with 4 weekly doses of 10-50 ug of C9F5 anti-idiotypic showed that by day 21 post-immunization there was about a 10-fold increase in the number of molecules of C3 per cfu. This level of C3 deposition was comparable to that obtained by immunizing with 1 ug of MEP. Lower (0.5 and 1 ug) amounts of the anti-idiotypic MAb achieved a 10-fold increase in molecules of C3 per cfu after the fourth immunization.

Detailed Description Text (38):

MEP, the major constituent of the slime that coats mucoid *P. aeruginosa*, appears to play a role in the ability of these mucoid bacteria to infect the lungs of CF patients. MEP may facilitate persistence of the organism in the lungs by blocking proper attachment of antibodies or complement. In addition, MEP is a heteropolymer, and antibodies to MEP may be directed to epitopes that do not promote efficient bacterial clearance via opsonophagocytic killing. Furthermore, antibodies of different isotypes, even when directed at the same epitope, may have different functional characteristics, some being deficient in opsonic activity. This phenomenon was seen here where the development of opsonic activity was associated with the appearance of IgG.sub.1, but not IgM, antibodies. Human trials with a purified MEP vaccine have demonstrated a disappointing opsonophagocytic-killing antibody response.

Detailed Description Text (39):

The present specification describes an anti-idiotypic antibody to an opsonizing MAb specific to MEP that immunologically mimics MEP in both in vitro and in vivo assays. The anti-idiotypic antibody binds to F(ab').sub.2 fragments from the opsonic anti-MEP MAb1, blocks binding of the same anti-MEP MAb1 to MEP, elicits MEP-specific antibodies in syngeneic and allogeneic mice, and binds to opsonic human antibodies obtained from MEP-vaccinated volunteers. Thus, these data support the belief that the anti-idiotypic MAb C9F5 functions as a molecular mimic of MEP. It is clear that anti-idiotypes can elicit antigen-specific antibody responses in vivo in the absence of actual native antigen.

Detailed Description Text (40):

Syngeneic BALB/cByJ mice receiving the C9F5 anti-idiotypic mimic of MEP made an isotypic response restricted to IgM antibodies. Such a restricted isotypic response resembles that seen to native polysaccharide antigens in which T cell help is presumably minimal. It seems possible that in the syngeneic system anti-idiotypes are seen primarily as "self" antigens poorly able to recruit T cell help.

Detailed Description Text (41):

The antibodies to MEP induced in allogeneic C3H mice by the anti-idiotypic were of both IgM and IgG.sub.1 isotypes. IgG1 subclass responses in mice are often elicited against T cell-dependent protein antigens. Since C3H mice differ from BALB/cByJ mice in MHC haplotype (H2.sup.k vs. H2.sup.d respectively), it is possible that presentation of the anti-idiotypic in this allogeneic system recruits T-cell help and an expansion of isotypes of the MEP-specific antibodies. The development of IgG antibodies in these studies is consistent with previous observations that development of IgG antibodies to opsonic epitopes on MEP is required for maximal phagocytic killing.

Detailed Description Text (42):

Of critical importance was the observation that the anti-idiotypic-induced, MEP-specific antibodies elicited in allogeneic mice fixed complement to the bacterial surface and had opsonic activity against mucoid *P. aeruginosa*.

Detailed Description Text (43):

It is believed that additional testing and screening in accordance with the procedures and techniques disclosed herein may be used to produce and isolate additional cell lines which produce antibody having similar qualities, characteristics and effectiveness as those disclosed herein.

Detailed Description Text (46):

It has been shown that the C9F5 anti-idiotypic generates protective immunity in mice, and that it works as a vaccine in mice. All data collected to date indicate that C9F5 will be efficacious in generating protective immunity to mucoid *P. aeruginosa* in humans and animals. Thus, it is believed that C9F5 anti-idiotypic, as a vaccine, will generate protective immunity and prevent infections from mucoid *P. aeruginosa* in humans, livestock, and animals. A vaccine induces protective immunity. A vaccine, when inoculated into or administered in an effective amount to the host, prevents infection by inducing production of protective antibodies and protective memory cells. The protective antibodies so produced can be collected and used as part of an immune or hyperimmune globulin. Such an immune or hyperimmune globulin can be passively administered to an individual in need of these protective antibodies.

Detailed Description Text (47):

To inoculate a human with C9F5 anti-idiotypic, it is believed that the proper dosage is about 50-100 ug, preferably about 100 ug, injected subcutaneously, generally in sterile saline or comparable solution. Typically, there would be two injections, one primary and one booster, separated by 4 to 8 weeks. Typically, the same amount is given in each

injection. Vaccination techniques and methodologies are known in the prior art, or may be readily adapted by one skilled in the art to enable use with the novel antibodies herein. The disclosures of U.S. Pat. Nos. 4,693,891 and 4,160,023 are incorporated herein by reference in their entirety. For effective treatment, it may be necessary to link the C9F5 to a carrier such as to conjugate the C9F5 anti-idiotype to another protein to enhance its immunogenicity, such as by covalent linkage to diphtheria toxoid or Pseudomonas exotoxin A. Covalent linkage to diphtheria toxoid and exotoxin A is known in the prior art. To those skilled in the art, similar inoculation techniques and dosage levels and schedules, based on experimental results, known proportions, ratios, and tendencies, would be utilized for inoculations in livestock and other animals.

Detailed Description Text (48):

A "pharmaceutical preparation" is used herein in its broader sense to include preparations containing a composition or antibody in accordance with the invention used not only for therapeutic or inoculation purposes, but also for reagent purposes as are known in the art or for tissue culture purposes. If the pharmaceutical preparation is to be employed as a reagent, then it should contain reagent amounts of composition or antibody. Similarly, when a pharmaceutical preparation is used in tissue culture or a tissue culture medium, it should contain amounts sufficient to obtain the desired growth.

Detailed Description Text (50):

C9F5 anti-idiotype can also be used as a substitute for MEP antigen for diagnostic purposes, such as detecting the presence of antibody in a specimen. For example, C9F5 anti-idiotype can be substituted for purified MEP antigen on an ELISA plate to detect anti-MEP antibody. C9F5 antibody can be coated onto or bound onto a substrate such as an ELISA plate. The specimen containing the antibody to be detected, such as anti-MEP antibody, is placed in contact with the bound C9F5. Anti-MEP antibody in the specimen then binds to the C9F5. Detection means are then employed to detect the presence of bound anti-MEP antibody. The procedure can also be reversed, whereby the specimen containing the antibody to be detected is applied to the ELISA plate first, and the C9F5 antibody is applied thereafter. Alternatively, the specimen can be bound to the substrate, such as nitrocellulose paper in a Western Blot, where the antibody to be detected can be separated out by such techniques as gel electrophoresis. A solution containing C9F5 antibody is then applied to the paper; the C9F5 binds to the bound antibody of interest and is then detected. Alternatively, radioactively-labeled C9F5 can be used using known techniques. The procedures and manipulative steps and techniques to be employed in these diagnostic methods correspond with those described herein or may be readily adapted or utilized by one skilled in the art to enable use with the novel antibodies herein.

Detailed Description Text (51):

It is believed that C9F5 will act much more specifically as a detector on such things as an ELISA plate than purified MEP antigen, since purified MEP typically is somewhat non-specific and binds to a greater variety of antibodies than does C9F5, including MEP antibodies that are nonopsonic and non-protective. C9F5 only binds to protective, opsonic antibodies and is thus a better measure of Pseudomonas immunity. The use of C9F5 anti-idiotype as a substitute for purified MEP antigen, both as a vaccine and as a diagnostic tool or probe, also has the advantage that the workers are working with and handling a non-toxic, essentially non-harmful protein such as C9F5, rather than working with, grinding up, handling, etc., the potentially pathogenic and hazardous mucoid P. aeruginosa bacteria. The safety of the workers is thus enhanced. It would also be much cheaper to use C9F5 anti-idiotypes, since they can be cheaply grown up in large quantities and more easily purified. Purified MEP is more expensive both to produce and purify.

Detailed Description Text (52):

C9F5 anti-idiotype, since it is monoclonal, can be used as a specific probe, using ELISA screening techniques, to determine how similar CF patients are, in that it can be determined whether different CF patients have raised up similar, or different, antibodies to mucoid P. aeruginosa. The C9F5 anti-idiotype would act as a probe for the heterogeneity of the variable region of the antibody to MEP. Determining the similarity or dissimilarity of CF patients, in the similarity or dissimilarity of their antibodies to P. aeruginosa, can be useful for diagnostic and research purposes.

Other Reference Publication (2):

Schreiber, J. R., et al., "Anti-Idiotype-induced Lipopolysaccharide-specific Antibody Response to Pseudomonas aeruginosa", The Journal of Immunology, pp. 188-193 (Jan. 1, 1991).

Other Reference Publication (3):

Schreiber, J. R., et al., "Anti-Idiotypic-Induced, Lipopolysaccharide-Specific Antibody Response to *Pseudomonas aeruginosa*", *Journal of Immunology*, vol. 144, pp. 1023-1029 (Feb. 1, 1990).

Other Reference Publication (4):

Pier, G. B., et al., "Opsonophagocytic Killing Antibody to *Pseudomonas aeruginosa* Mucoid Exopolysaccharide in Older Noncolonized Patients with Cystic Fibrosis", *The New England Journal of Medicine*, vol. 317, pp. 793-798 (Sep. 24, 1987).

Other Reference Publication (5):

Dressman, G. R. and Kennedy, R. C., "Anti-Idiotypic Antibodies; Implications of Internal Image-Based Vaccines for Infectious Diseases", *The Journal of Infectious Diseases*, vol. 151, pp. 761-765 (May, 1985).

Other Reference Publication (6):

Westerink, M. A. J., et al., "Development and Characterization of an Anti-Idiotypic Antibody to the Capsular Polysaccharide of *Neisseria meningitidis* Serogroup C", *Infection and Immunity*, vol. 56, pp. 1120-1127 (May, 1988).

Other Reference Publication (7):

Percival, D. A., et al., "Anti-Idiotypic Antibody-Induced Protection Against *Clostridium perfringens* Type D", *Infection and Immunity*, vol. 58, pp. 2487-2492 (Aug., 1990).

Other Reference Publication (11):

Pier, G. B., et al., "Suppression of the Opsonic-Killing Antibody Response to *Pseudomonas aeruginosa* Mucoid Exopolysaccharide by Anti-Idiotypic Cytotoxic T Cells", *Clinical Research*, vol. 38, p. 390A (Apr., 1990).

Other Reference Publication (12):

Schreiber, J. R., et al., "Characteristics of a Pilot Anti-Idiotypic *Pseudomonas* Vaccine; Isotype and Function of Anti-Idiotypic-Induced Antibodies", Fourth Annual North American & 1990 International Cystic Fibrosis Conference Abstract, Arlington, Va., Oct. 3-6, 1990.

CLAIMS:

1. An anti-idiotypic monoclonal antibody or a binding fragment thereof, said monoclonal antibody or binding fragment thereof being produced by the cell line designated C9F5 and having ATCC Accession No. HB 10715 or a subclone thereof having all the properties and characteristics of the cell line having ATCC Accession No. HB 10715.
2. A composition comprising an anti-idiotypic monoclonal antibody or binding fragment thereof according to claim 1, and an acceptable carrier.

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DOCUMENT-IDENTIFIER: US 5998381 A
TITLE: Compounds that bind bacterial pili

Drawing Description Text (2):

FIG. 1A schematically shows steps in the formation of intermediates in the design of an antibiodiotoxic of the present invention. FIG. 1B schematically shows the final step in forming the antibiodiotoxic from the intermediates of FIG. 1A. FIG. 1C schematically shows the beginning steps in the formation of intermediates in the design of another antibiodiotoxic of the present invention. FIG. 1D shows the steps in forming the antibiodiotoxic from the dialdehyde of FIG. 1C.

Drawing Description Text (5):

FIG. 4 describes solid phase assays for determining the level of binding of antibiodiotoxins of the present invention. Step 1 shows toxin or organisms in a testing microwell. Step 2 schematically represents the binding of antibiodiotoxic. Step 3 schematically shows the binding of secondary reagents.

Detailed Description Text (22):

The present invention also relates to therapeutics for the prevention and treatment of blood-borne and toxin mediated diseases, and in particular the prevention and treatment of sepsis caused by various types of organisms in humans as well as other animals. The present invention is particularly suited for the in vivo neutralization of the effects of endotoxin. However, it is contemplated that the present invention will be used in the treatment of gram-negative and gram-positive sepsis. Although the invention may be used for treatment of sepsis due to one organism, it may also be used to treat sepsis caused by multiple organisms (e.g., sepsis and/or bacteremia due to gram-negative and gram-positive organisms). The present invention also contemplates treatment comprising multiple antibody-antibiotic conjugates used in combination. It is also contemplated that the present invention will be used to treat bacteremia, viremia or fungemia, by enhancing the removal of organisms by opsonization.

Detailed Description Text (23):

In accordance with the present invention, soluble antibody-antibiotic conjugates or "antibiodiotoxins" are administered intravenously, intra-muscularly, subcutaneously, intradermally, intraperitoneally, intrapleurally, intrathecally or topically. The conjugate is water-soluble if it has a solubility in physiologic saline of at least 0.1 mg/ml, and preferably of at least 1.0 mg/ml, when measured at room temperature. The present invention contemplates the use of antibiodiotoxins in a therapeutic preparation for both prophylactic and acute treatment.

Detailed Description Text (24):

While the benefit conveyed by treatment according to the present invention is not dependent on the understanding of the mechanism(s) by which soluble antibody-antibiotic conjugates achieve a therapeutic result, it is believed that, in the case of bacteria, success is accomplished by: (i) binding and opsonization of bacteria; (ii) bacterial killing (direct killing by the conjugate and/or complement-mediated); and (iii) neutralization and removal of free bacterial toxins (e.g., gram-negative endotoxin, thereby preventing initiation and/or escalation of the septic reaction).

Detailed Description Text (27):

The description of the invention involves: (I) Antibiodiotoxic Design and Characterization; (II) Antibiodiotoxic in vitro and in vivo Efficacy; (III) Antibiodiotoxic Applications; and (IV) Therapeutic Preparations and Combinations. Section III describes the use of antibiodiotoxins for: (A) Prophylactic Use in Humans; (B) Acute Therapy in Humans; and (C) Veterinary Care.

Detailed Description Text (28):I. Antibiotic Design and CharacterizationDetailed Description Text (29):A. AntibodiesDetailed Description Text (30):

In designing antibiotics, all types of antibody (e.g., IgG, pentameric and monomeric IgM, secretory and monomeric IgA, IgE and IgD) are contemplated. Nonetheless, there are advantages to using a particular class of antibody. Table 1, for example, compares the characteristics of IgG and IgM. While IgM has the advantage of better opsonization and complement activation, IgG has a longer half-life in vivo and can be raised to higher titers because of the fact that it is the primary antibody raised during secondary responses to antigen. Consequently, the preferred antibody for conjugation according to the present invention is IgG.

Detailed Description Text (31):

While antigen-specific IgG can be employed (e.g., bacteria-seeking antibodies), antigen-specificity may result in a shorter half-life of the compound (and/or greater cost). Consequently, the preferred antibody is non-specific. [Contrast C. H. J. Ford et al., Indian J. Pediatr. 57:29-46 (1990).]

Detailed Description Text (32):

Goers et al. (U.S. Pat. No. 4,867,973) describe the use of antibody conjugated to antimicrobials, but with antigen-specific antibody. In contrast, the conjugates of the present invention utilize non-specific antibody. Goers et al. describe in particular the conjugation to antigen-specific monoclonal antibodies. Monoclonal antibodies have not been a step forward in the prevention and/or treatment of bacteremia and sepsis. While these preparations should possess greater potency and specificity than polyclonal sera, they are: a) prohibitively expensive; b) frequently immunogenic; and c) exhibit unusually short circulating half-lives (typically less than 24 hours).

Detailed Description Text (33):

With respect to cost, Centoxin (a commercially produced antigen-specific monoclonal antibody) serves as a real life example; the price was approximately \$3,700.00 per 100 mg dose. Pharmacoeconomic analysis indicated that--even if the product was used under strict guidelines for acute cases--"its use could add \$2.3 billion to the nation's health care budget." [K. A. Schulman et al., JAMA 266:3466-3471 (1991).] The expense of Centoxin is such that it simply could not be used prophylactically. The conjugates of the present invention, on the other hand, are produced from materials costing a fraction of this figure (e.g., \$2.00 per 100 mg dose) because of the readily available inexpensive source of pooled donor IgG.

Detailed Description Text (34):

Also, human monoclonals while perhaps lessening the chance of immunogenicity, do not overcome the problem of short circulating half-lives. In a study using human monoclonal anti-lipid A antibody in patients with sepsis syndrome, the mean serum half-life was approximately sixteen (16) hours. [See C. J. Fisher et al., Clin. Care Med. 18:1311-1315 (1990).] To maintain a protective level of antibody, this reagent would need to be given repeatedly. Again, the cost of such an approach would be staggering.

Detailed Description Text (35):

From the above, it should now be clear why the limitation to "non-specific immunoglobulin" is a critical limitation that is unique to the present invention. Non-specific IgG is easily and cheaply obtained, requiring no immunization and eliciting no immune response in a syngeneic setting. Non-specific IgG does not have the standardization problems of antigen-specific antibody. Simply put, there is no antigen-specific titer to be concerned about (let alone variability in the titer from unit to unit). Rather, standardization comes from the conjugated ligand; conjugation of non-specific IgG results in >1000-fold increase in LPS-binding titer and by standardization of the ligand that is attached, one standardizes the activity of the therapeutic. Finally, non-specific IgG, unlike monoclonals, has a long half-life needed for a prophylactic (compare the >21 day half-life of pooled polyclonal human IgG with the mean serum half-life of 16 hours for the human monoclonal antibodies discussed above).

Detailed Description Text (39):

The commercial product displays a broad spectrum of opsonic and neutralizing antibody

activities. When administered intravenously, essentially 100% of the infused IgG antibodies are immediately available in the recipient's circulation. The in vivo half-life equals or exceeds the three week half-life reported for IgG in the literature. It is therefore quite acceptable for use in the preparation of antibody-antibiotic conjugates of the present invention.

Detailed Description Text (40):

Of course, the infusion of large amounts of antibody in humans is contraindicated in individuals who are known to have had previous anaphylactic or severe systemic responses to IgG. Care must also be taken to confirm that there is no sensitivity to the trace amounts of other antibody (e.g., IgA).

Detailed Description Text (41):

Before administration of the antibody-antibiotic conjugates of the present invention to humans, it may be good medical practice to have an antibodiotic sensitivity test performed. This can be done by subcutaneously injecting a small amount of the conjugate in the arm of the patient. A salt solution is injected in the other arm as a control. Normally, a positive hypersensitivity test is indicated by no more than formation of a welt on the skin surface with surrounding swelling. Some patients, however, develop anaphylactic shock (i.e., a full-blown immediate hypersensitivity reaction). It is recommended that adrenalin be available for these cases.

Detailed Description Text (46):

In the design of antibody-antibiotic conjugate, a primary consideration is the mode of action of the antibiotic. Since the conjugates will be much larger molecules than the parent antibiotics, only antibiotics that bind to exposed or secreted components (e.g., toxins) of the bacteria, fungus, virus, or parasite are likely to target the antibody carrier to the pathogen or its products. For example, penicillin antibiotics disrupt bacterial cell wall synthesis and bind to surface-exposed components of certain bacteria whereas aminoglycoside antibiotics commonly bind to ribosome subunits in the cell cytoplasm. The former is a much better candidate for effective antibody-antibiotic conjugates than the latter.

Detailed Description Text (47):

Antibiotics vary greatly in the type and species of organisms upon which they are active. For example, certain antibiotics such as the polymyxins are far more effective against gram-negative bacteria, whereas other antibiotics such as vancomycin tend to be more effective against gram-positives. Some, like the cephalosporins, and broad-spectrum penicillins are comparably effective against both types. Other antibiotics, such as amphotericin are primarily antifungal agents whereas amantadine exhibits activity against certain influenza viruses. In designing antibody-antibiotic conjugates for the prevention or treatment of disease one must consider the spectrum of antibiotic activity desired and select those antibiotic(s) that are active against the target pathogen(s) and, as described above, act primarily on exposed components of the pathogen(s).

Detailed Description Text (49):

Within a family of antibiotics (e.g., penicillins, cephalosporins, polymyxins) there are structural features common to all members. However, there often exists a wide variety of natural and synthetic variations on this common structure that may influence the activity spectrum, pharmacokinetics, or other properties of the antibiotic. In the design of antibody-antibiotic conjugates, these structural differences within an antibiotic family are important from two perspectives. First, the activity spectrum may influence the choice of antibiotic; and, second, the chemical differences between antibiotics will influence the range of cross-linking chemistries available to conjugate the antibiotic. For example, the variable side chain component of penicillin antibiotics is a methyl benzyl group in penicillin G but the variable side chain group is a phenolic group with a primary amine side chain in amoxicillin. The latter antibiotic presents a wider array of potential modes for cross-linking than does penicillin G.

Detailed Description Text (52):

The level of protection achieved by the present invention is best understood when compared with other known approaches (see Table 3). For example, the widely-tested and publicized monoclonal antibody Centoxin-HA-1A is capable of binding endotoxin and neutralizing its biological activity. However, when compared to an IgG-PMB conjugate of the present invention, the monoclonal antibody is costly and suffers from low affinity and short half-life. The latter characteristics may explain why the human clinical studies have yet to yield clear benefits.

Detailed Description Text (53):

Others have attempted to reduce the toxicity of polymyxin B by attachment to dextran. [D. A. Handley, Eur. Patent Appl. Pub. No. 428486.] However, dextran has a half-life in humans of only about a day. By use of immunoglobulin according to the present invention, a much longer half-life is achieved (see Table 4 and Examples 24 and 25). Dextran, having no Fc receptor (FcR), also has no known capacity to promote opsonization or activate complement (C').

Detailed Description Text (63):

Numerous agents have been developed for the cross-linking of biological molecules. [Pierce Chemical Co., (Rockford, Ill.), General Catalog, pp. E-10-E-39 (1992).] In general, these agents possess functional groups that are reactive with the side chains of different amino acids found in proteins or peptides. As summarized in Table 5, various functional groups will react with primary amino groups, carboxyl groups, hydroxyl groups, or thiol groups of proteins or other compounds. In the design of antibody-antibiotic conjugates, the reactive groups of both the antibody and antibiotic must be considered. In general, antibodies have many reactive groups that can be used in direct conjugation schemes (amino acids containing primary amine, carboxyl, hydroxyl, thiol [after reduction]) or modified groups (glycosylated amino acids that can be oxidized to aldehyde; or primary amines that can be made thiol-reactive) for conjugation schemes. Individual antibiotics will not, in general, possess very many different reactive groups and offer fewer choices for conjugation to antibodies. The selection of an antibiotic from a family of related compounds and the selection of a cross-linking scheme must take into consideration the reactive groups on an antibiotic.

Detailed Description Text (65):

Different cross-linkers may influence the activity of individual antibiotics and the efficiency with which they are conjugated to antibodies. In the design of antibody-antibiotic conjugates, the discovery of more optimal cross-linkers relies on the empirical analysis of conjugates prepared using varying concentrations of different cross-linkers.

Detailed Description Text (66):

The in vivo safety and efficacy of antibody-antibiotic conjugates will depend upon their activity, toxicity and stability. The selection of the cross-linking agent may also affect these aspects of conjugate performance. For example, in addition to influencing the activity of the conjugate imparted by the antibiotic, the cross-linker employed may affect the properties of the antibody. Effector functions dependent upon the Fc region of the antibody such as opsonization or complement fixation may be influenced by which reactive groups are utilized and their location on the antibody molecule. Furthermore, some cross-linkers may cause adverse reactions by eliciting an immune response to the haptenic groups on the cross-linker. Finally, the in vivo stability of the bonds created by the cross-linking scheme may vary in important ways. Disulfide bonds linking the antibiotic and antibody may not be as stable, for example, as amide bonds created by other cross-linkers. Dissociation between antibody and antibiotic may not be tolerable in cases where long-term prophylaxis is desired.

Detailed Description Text (68):

The present invention contemplates the use of antibody analogues. Antibody analogues are those compounds which act in an analogous manner to antibodies. In one embodiment, the present invention contemplates fragments of antibodies (e.g., Fc fractions) to make antibody-antibiotic conjugates. As herein used, the terms "antibody" and "immunoglobulin" are meant to include antibody analogues.

Detailed Description Text (69):

The present invention also contemplates the use of fusion proteins containing antibody effector sites as applied to the Immunobiotic.TM. and Immunoadapter.TM. approaches. Specifically, recombinant proteins representing human antibody sequences can be employed as a framework to attach targeting ligands.

Detailed Description Text (71):

Antibiotic compounds have been isolated from many different microbial, plant, and animal sources and new promising compounds continue to be discovered. In addition, synthetic derivatives of natural compounds as well as wholly synthetic compounds such as small peptides are also being screened for antibiotic activities in many laboratories. As used herein, the term "antibiotic" refers to any chemical compound which destroys, inhibits the growth of, or binds to microorganisms (i.e.,

"antimicrobials"). It is not intended that the term be limited only to those compounds which are produced by microorganisms. "Antibiotic" therefore includes compounds which are produced synthetically, as indeed many of the antibiotics are now produced in the chemistry lab rather than by microorganisms. Polymyxin and other compounds discussed herein may be produced synthetically or obtained from "natural" sources (e.g. B. polymyxa). Therefore, the invention contemplates the design and synthesis of a variety of antibody-antibiotic conjugates utilizing antibiotics from all sources.

Detailed Description Text (76):

Mode III: Check specificity of the antibodiotic by inhibition of bacterial toxin binding with the antibiotic.

Detailed Description Text (77):

Mode IV: Assess the antibodiotic for inhibition of organisms growth in liquid culture.

Detailed Description Text (79):

For example, antibiotic X may initially be evaluated by Mode I. In this Mode, X is only conjugated to a cross-linker "c" to create "X-c"; this compound is then added to a liquid or solid phase culture. By creating only part of antibodiotic, the question of compatibility with immunoglobulin is avoided; Mode I only addresses compatibility of "X" with the conjugation chemistry. The assay is performed and the results are compared to an identical assay of unconjugated antibiotic X.

Detailed Description Text (83):

If the activity of X-c is good, it is further evaluated in Mode IIA. If the activity of X-c is poor, X is evaluated in Mode IIB. Both Modes IIA and IIB contemplate covalent attachment; Mode IIA uses a cross-linker to create "X-c-Ig", while Mode IIB does not use a cross-linker and generates "X-CHO-Ig." In both cases, the antibody-antibiotic conjugate, or simply the "antibodiotic", is assayed on a solid phase assay such as shown schematically in FIG. 4.

Detailed Description Text (84):

Toxin or organisms may be used in the solid phase assay to coat a microwell or other appropriate surface (Step 1, FIG. 4A). The antibodiotic is then added to test for binding (Step 2, FIG. 4A). Standard washing procedures are used to avoid non-specific binding. The antibody portion of the conjugate may thereafter serve as a target for secondary reagents (e.g., goat anti-human IgG antibody having an enzyme reporter group such as horseradish peroxidase) (see Step 3, FIG. 4A). An appropriate substrate for the enzyme may then be added (not shown) to generate a colorimetric signal.

Detailed Description Text (87):

The next portion of the evaluation involves testing the antibody-antibiotic conjugate for growth inhibition and/or bactericidal activity (Mode IV). This is the same assay as shown in FIG. 2, the difference being that now the complete conjugate X-c-Ig (or X-CHO-Ig) is evaluated rather than just the antibiotic (X-c).

Detailed Description Text (89):

Thoughtful consideration of the results of each of these steps allows any antibiotic to be analyzed for potential use in the form of an antibodiotic. Following these in vitro tests, the antibiotic can then be evaluated in vivo for reduced toxicity and pharmacokinetics. "antibody" and "immunoglobulin" are meant to include antibody analogues.

Detailed Description Text (93):

The present invention also links antibiotics non-covalently with the potent effector functions of antibodies. In this manner, the antibiotics serve as high affinity surface binding ligands. A hybrid molecule is created with antimicrobial properties superior to antibodies generated through natural infection or vaccination.

Detailed Description Text (94):

The Immunoadapter.TM. format takes advantage of ligand targeting via a non-covalent link to the antibody through the antigen combining site. This is accomplished with Immunoadapter.TM. compounds that consist of the surface targeting ligand (e.g. antibiotic) attached to a hapten that will bind to specific antibodies. This format has several engineering and therapeutic advantages. Small molecule targeting ligands can be engineered using synthetic organic chemistry independently of the antibody protein (thus avoiding the problem of damage to the antibody by contact with organic solvents). This flexibility in chemical synthesis allows a limitless range of small molecule and peptide ligands to be used.

Detailed Description Text (95):

This format is contemplated for both active and passive immunotherapy regimens. In the active mode, the patients circulating antibodies (either naturally-occurring or those resulting from vaccination) can be directed to microbes via the Immunoadapter.TM. compound. This avoids the cost, efficacy and safety concerns when using donor antibodies. Passive hapten-specific antibody may be optimal in some cases, and can be obtained from specific donor, cell culture or recombinant sources.

Detailed Description Text (98):

linking chemistries--choice of reactive groups, efficiency of reactions, placement on antibody, length of spacer arms, novel reactive groups

Detailed Description Text (99):

antibody compositions--retention of effector functions, solubility and serum half-life

Detailed Description Text (102):

II. Antibiotic In Vitro and In Vivo Efficacy

Detailed Description Text (104):

In the previous section, the key question was whether the antibiotic portion of the conjugate shows the same or similar reactivity as the native antibiotic. However, it must be emphasized that immunoglobulin is not simply an inert carrier. The Fc portion of the antibody can mediate pathogen elimination by two mechanisms that are distinct from the effects of the antibiotic. First, it is known that following binding of antibody to antigen, the Fc region can activate the classical pathway of complement, ultimately resulting in the lysis of organisms. Second, binding of the conjugate to bacteria can lead to the ingestion or opsonization of the organism by recognition of the Fc region by phagocytes (e.g., macrophages) and/or lysis by killer cells. [See L. E. Hood et al., Immunology, 2d Ed., The Benjamin/Cummings Publishing Company, Inc., Menlo Park, pp. 339-340 (1984).]

Detailed Description Text (105):

The present invention contemplates antibody-antibiotic conjugates with the capability of binding Fc receptors on phagocytes. It is preferred that in competition binding, the binding of the antibody-antibiotic conjugates of the present invention to such cells is substantially similar to that of normal IgG.

Detailed Description Text (106):

The present invention contemplates antibody-antibiotic conjugates which, while not activating complement systemically, are capable of binding complement to facilitate pathogen killing. Furthermore, conjugates are contemplated which bind phagocytes via the Fc region to facilitate pathogen elimination. Thus, it is contemplated that the antibody-antibiotic conjugates will mediate or enhance opsonization and removal (opsonophagocytosis) of the etiologic agent(s) of sepsis in the treated patient.

Detailed Description Text (108):

Regardless of the manner in which the conjugate is used in vivo (acute, prophylactic, etc.), the conjugate will be present in a background of the entire repertoire of host immune mediators. These immune mediators include, of course, humoral immune mediators such as endogenous antibodies directed against bacteria and their toxins.

Detailed Description Text (110):

These studies suggest that patients at risk of gram-negative sepsis and endotoxemia may be so because of weakened humoral immune defenses. For this reason, the present invention contemplates, in one embodiment, determining the immune status of the host prior to administration of the antibiotic. This determination can be made by screening potential risk groups for total and endotoxin core antigen-specific IgG and IgM levels. [B. J. Stoll et al., Serodiagnosis and Immunotherapy 1:21-31 (1987).] Screening is believed to be particularly important with the elderly, full-term and pre-term neonates [W. Marget et al., Infection 11:84-86 (1983)], patients with malignancies [C. Stoll et al., Infection 13:115-119 (1985)], abdominal surgery candidates, individuals under long-term catheterization or artificial ventilation, and burn and other trauma victims.

Detailed Description Text (111):

Where the immune status is poor (e.g., low total IgG levels and low levels of anti-bacterial antibodies), the efficacy of the antibody-antibiotic conjugate is expected to be most dramatic. Where the host's immune status is good, use of the

conjugate will support the endogenous anti-bacterial defenses.

Detailed Description Text (112):

For optimal in vivo treatment, the conjugate itself must be effective against clinically relevant organisms, non-toxic and non-immunogenic. Thus, it is contemplated that the conjugates of the present invention will be effective against gram-positive and gram-negative organisms which are commonly associated with sepsis (e.g., E. coli, K. pneumoniae, P. aeruginosa, S. pyogenes, S. aureus, S. epidermidis, etc.). It is also contemplated these conjugates will be non-toxic to the host animal. As with any chemotherapeutic, the conjugate must be effective against the infecting organisms, but not harm the host. In addition, in order to enhance the host's response to the infecting organism and to prevent such complications as serum sickness upon subsequent administration of conjugate, the conjugates themselves must be non-immunogenic. This characteristic permits the immune system of the host to focus on battling the infecting organisms, rather than attack the conjugates intended as treatment. As it is contemplated that these conjugates may be administered to the same animal multiple times (i.e., upon subsequent exposures to potentially pathogenic organisms) it is important that the host not produce antibodies against the conjugates themselves. Such antibody production would be likely to lead to rapid clearance of the conjugate upon subsequent administration or result in a serious, potentially life-threatening hypersensitivity response.

Detailed Description Text (113):

Conjugates which are non-immunogenic or poorly immunogenic due to high concentrations of D-configuration amino acids are also contemplated. Synthetic polypeptides entirely comprised of D-amino acids are generally unable to elicit an immune response. [M. Sela, in Advances in Immunology, Vol. 5, (F. Dixon and J. Humphrey, eds.), pp. 29-129 (1966).] Thus, conjugation of a synthetic antimicrobial comprised entirely of D-amino acids to the antibody would be beneficial in the present invention.

Detailed Description Text (114):

III. Antibiodiolic Applications

Detailed Description Text (122):

Rather than the short (i.e., three to seven day) period of protection provided by native antibiotics, the use of the antibiotic-antibody conjugates of the present invention should protect the trauma patient during the entire period of risk.

Detailed Description Text (130):

As noted previously, the present invention also contemplates the use of antibiodiols in a therapeutic preparation for acute treatment. In this case, treatment involves administration of the antibody-antibiotic conjugates after infection is detected and/or sepsis is suspected.

Detailed Description Text (141):

The present invention contemplates the use of the antibody-antibiotic conjugates of the invention (e.g., antibody-polymyxin conjugates) as "endotoxin sponge(s)," i.e., compounds which bind and facilitate clearance of released endotoxin. In a preferred embodiment, it is contemplated that the conjugates will be administered prior to the administration of standard antibiotics, both in order to bind the endotoxin released during microbial growth, as well as a preparatory step which will permit aggressive antimicrobial therapy without causing adverse effects due to endotoxin release following cell death. In this manner, the conjugates will be administered in conjunction with antimicrobial treatment. Importantly, it is contemplated that the endotoxin sponge will be safely administered intrathecally or intravenously, in order to directly place the compound in the needed location.

Detailed Description Text (142):

In addition, the present invention contemplates the treatment of meningitis caused by gram-positive organisms. In this regard it is contemplated that certain conjugates will again act as an endotoxin sponge, enhancing the clearance of any endotoxin present in the blood and/or CSF due to increased bowel mucosa permeability. It is also contemplated that certain conjugates (e.g., vancomycin-antibody conjugates) will effectively remove analogous substances produced and released by gram-positive organisms. For example, a "capsular sponge" would be useful for binding and clearing the large amount of capsular material produced by Streptococcus pneumoniae. This embodiment would also enhance opsonophagocytosis, as the antiphagocytic activity of the capsule will be neutralized by the capsular sponge.

Detailed Description Text (144):

Septicemia and sepsis are by no means limited to human beings. Infection by gram-negative bacteria accounts for significant morbidity and mortality in neonatal livestock, such as calves. [D. D. Morris et al., Am. J. Vet. Res. 47:2554-2565 (1986).] Interestingly, humoral immune status is again related to susceptibility to sepsis and this is largely dependent on passive transfer from colostrum. For this reason, the present invention contemplates, in one embodiment, determining the immune status of the animal prior to administration of the antibiotic. This determination can be made by screening neonatal calves for total circulating serum immunoglobulin (e.g., by ELISA).

Detailed Description Text (145):

Where the immune status is poor (e.g., low total IgG levels), the conjugate should be used prophylactically. Where the animal's immune status is good, use of the conjugate may be needed for acute therapy of gram-negative bacterial sepsis, which remains prevalent in neonatal calves even with high antibody levels.

Detailed Description Text (151):

With respect to the mode of administration, the antibiotics may be employed for intravenous, intramuscular, intrathecal or topical (including topical ophthalmic) administration. Formulations for such administrations may comprise an effective amount of antibiotic in sterile water or physiological saline.

Detailed Description Text (155):

Where repeated administrations are required, it may be beneficial to first clear any anti-hapten antibodies by administering free antibiotic. This can then be followed by administration of the antibiotic.

Detailed Description Text (157):

The present invention contemplates three strategies to create anti-infectives with the effector functions and long serum half-life of immunoglobulin G and a spectrum of pathogen reactivity determined by coupling or complexing microbe-binding ligands to complete immunoglobulins or immunoglobulin fragments. The preferred embodiments employ the properties of the IgG1 subclass, i.e., a serum half-life of 21-23 days in the average patient, complement activation and opsonization mediated by the binding of immunoglobulin to receptors on phagocytes. Therefore, like natural IgG1, these novel anti-infectives will have the ability to induce both the killing of pathogens and the removal of toxic debris. The long serum half-life is desirable because a single dose is then needed for extended prophylaxis. Low cumulative dosing, constant serum concentrations, and conjugation may also reduce the risk of certain toxicities associated with some ligands. The fact that IgG1 does not distribute to the GI tract is also desirable because resistance to many anti-infectives, particularly antibiotics, often arises in the flora of the GI tract.

Detailed Description Text (160):

The synthesis of Immunobiotics.TM. compounds by the genetic fusion of peptides that bind to microbial surfaces with fragments of IgG by methods that retain the Fc receptor-binding and complement-activating effector functions of the IgG is also contemplated. These constructs are also referred to as Artificial Antibodies.

Detailed Description Text (161):

The synthesis of Immunoadapter.TM. compounds to which immunoglobulin is non-covalently linked and thereby targeted to a pathogen. Immunoadapter.TM. compounds comprise surface targeting ligands attached to haptens that will bind to specific antibodies.

Detailed Description Text (162):

When the surface-binding ligands are antibiotics, the Immunoadapter.TM. compounds will have bactericidal/bacteriostatic activity that is independent of immune effector functions. The use of surface-binding ligands to target immunoglobulin effector functions will broaden the repertoire of binding specificities and affinities beyond what can be obtained with natural antibodies. With this targeting it may be possible to 1) produce a highly potent immunoglobulin formulation specific to a molecular target that is poorly immunogenic, 2) develop linkers which allow the ligand to access molecular targets in structures inaccessible to antibodies, 3) select ligands with very high target affinity and 4) select ligands with very broad target specificity. In addition, efficiencies may be gained in discovery and product development by selecting available ligands of suitable and well characterized specificity and affinity rather than by trying to isolate a natural antibody to meet specifications. One such specification is an anti-infective with immune effector functions that binds to a molecular target that is highly conserved in Gram-negative or Gram-positive bacteria or

all eubacteria. Such compounds or formulations can be used for broad spectrum prophylaxis or therapy of infectious diseases and would present a high barrier to the emergence of resistant organisms.

Detailed Description Text (163):

In one embodiment, an Immunoadapter.TM. compound is constructed by covalently linking polymyxin B to fluorescein. Polymyxin B serves to bind the Immunoadapter.TM. compound to Gram-negative organisms. The fluorescein is a nontoxic hapten that can be bound non-covalently by the antigen recognition site of monoclonal or polyclonal anti-fluorescein antibodies. In principle, fluorescein can be replaced by other hapten-antibody pairs, or the same anti-fluorescein antibody can be directed to different spectra of pathogens by altering only the ligand and linker.

Detailed Description Text (165):

In one embodiment, the Immunoadapter.TM. compound and immunoglobulin are mixed before administration and complexes are formed by hapten-antibody interaction (i.e. noncovalent binding). These complexes are expected to display the pharmacokinetic properties of their corresponding IgG components. After an intravenous injection, the concentrations of the complexes in serum and extravascular compartments will take more than a day to equilibrate, and the serum half-life should approach 3 weeks.

Detailed Description Text (166):

In another embodiment, the Immunoadapter.TM. compound may be administered alone followed by a "chasing" dose of antibody. This regimen may be appropriate when the ligand is an antibiotic and high doses with rapid distribution are needed to suppress an infection. The "chasing" antibody may be complexed with the same ligand and can be administered in a lower concentration for secondary prophylaxis. Alternatively, an uncomplexed antibody can be administered to clear ligand-bound pathogen debris.

Detailed Description Text (167):

The present invention also contemplates the creation of Immunoadapter.TM. compounds with ligands capable of binding to a desired target and haptens that can be recognized by antibodies endogenous to the patient. By converting the specificity of some high titer pool of hapten-specific antibodies in the patient, one can overcome the shortcomings of passive immune strategies. The cost of providing an antibody carrier is either eliminated or replaced by the cost of a vaccine to boost the titers of an endogenous antibody pool. The antibodies will already be distributed to tissues and the Immunoadapter.TM. compound, if relatively small, will be able to rapidly distribute to tissues.

Detailed Description Text (168):

There are at least three specifications for selecting a hapten-specific pool of antibodies. The antibodies must: possess a reasonable affinity for the hapten; not be needed for protection against any immediate threat, i.e., conversion of specificity should not put the patient at risk; be in sufficient concentrations that when complexed with Immunoadapter.TM. compounds they can provide protection against the target pathogens. Examples of preferred haptens include, but are not limited to, dinitrophenol, haptens recognized by antibodies to blood group antigens.

Detailed Description Text (169):

The present invention contemplates the employment of vaccination to obtain high titers of hapten-specific antibodies. The vaccine can be entirely novel, for example the antigen can be a fluorescein hapten coupled to a carrier (e.g. a carrier such as tetanus toxoid). With this approach one can design haptens that are unlikely to interfere with the patient's humoral defenses. An alternative is to use haptens in the Immunoadapter.TM. compounds that are immunodominant epitopes of antigens in mandated or widely used vaccines (e.g., tetanus toxoid itself). By this approach, the patient may not need a booster vaccination or may only require only one booster vaccination just prior to entering a period of risk in which Immunoadapter.TM. compounds will be used for protection.

Detailed Description Text (172):

The technology can be employed for the treatment, primary prophylaxis, or secondary prophylaxis of infectious diseases in many clinical or community situations. The technology is designed particularly for situations where it is known in advance that the patient will be at risk for a defined and limited duration. For example, oncology patients receiving cytotoxic chemotherapy are at high risk of infection due to a transient neutropenia that is most severe for a few days in the middle of the recovery phase of each cycle. Vaccination 2-3 weeks prior to scheduled chemotherapy should

induce carrier antibody titers sufficient to provide protection during the recovery cycle. Immunoadapter.TM. compounds can be administered with chemotherapy, and an uncomplexed antibody pool may be replenished during the recovery phase. The replenished uncharged pool and a new dose of Immunoadapter.TM. compounds can be used for protection during the next cycle of chemotherapy.

Detailed Description Text (173):

Another example involving advanced notice are travelers requiring temporary protection against pathogens that are problems in certain geographical areas. Where insufficient lead time exists for conventional vaccination, an Immunoadapter.TM. compound may be given that is reactive with high titers of endogenous hapten-specific antibody.

Detailed Description Text (174):

Finally, rapid military deployment is a good example where the specific need may be unanticipated but there is a routine vaccination to maintain titers of anti-hapten antibodies that can be complexed with a hapten that is generic to Immunoadapter.TM. compounds with different target specificities.

Detailed Description Text (175):

It may be desirable to 1) determine the patient's endogenous levels of antibody prior to treatment and 2) monitor serum concentrations and clearance of complexed antibody. The following formats may be employed for this purpose when fluorescein is used as a hapten:

Detailed Description Text (176):

a) Concentrations of uncharged antibody can be determined by adding Immunoadapter.TM. compound to a sample of serum and measuring the fluorescence polarization.

Detailed Description Text (178):

fluorescence polarization after the addition of anti-target ligand antibody

Detailed Description Text (179):

an ELISA in which the Immunoadapter.TM. compound is captured by an anti-ligand antibody adsorbed to the plate and detected by an anti-fluorescein antibody that is either labeled or used with a secondary anti-Fc antibody that is labeled.

Detailed Description Text (180):

c) Concentrations of complexed hapten-specific antibody can be determined by an ELISA in which the complex is captured by anti-ligand antibody adsorbed to a plate and detected by an anti-Fc antibody that is labeled.

Detailed Description Text (181):

The potential pharmaceutical applications for Immunoadapter.TM. technology go beyond the scope of infectious disease. For instance, antibodies could be directed to molecular targets on normal or neoplastic cells of the patient that are poor immunogens. Also, while infectious diseases are generally acute, Immunoadapter.TM. technology need not be restricted to acute care. For example, the present invention contemplates reagents that might be used to induce the phagocytic clearance of amyloid deposits.

Detailed Description Text (188):

This example describes attempts to attach antibiotics to a carrier (i.e., in this case antibodies). In this regard, K. Hanasawa et al. describe the attachment of PMB to an immobilized fiber via carbodiimide chemistry. [Surg. Gyn. & Ob. 168:323-331 (1989).] In this example, the ability of a carbodiimide cross-linker to conjugate polymyxin B (PMB) to human IgG was analyzed.

Detailed Description Text (195):

In order to detect bound antibodies, the wells incubated with the human antibody conjugates were incubated with 100 .mu.l of a 1:500 dilution of goat anti-human IgG (whole molecule)-alkaline phosphatase conjugate (Sigma) and the wells incubated with the rabbit serum were incubated with 100 .mu.l of a 1:500 dilution of goat anti-rabbit IgG (whole molecule)-alkaline phosphatase conjugate (Sigma) for 2 hours at room temperature. The secondary antibody solutions were discarded, the plates were washed with BBS-Tween 20, and PBS-Tween 20 as above and then twice with 50 mM Na.sub.2 Co.sub.3, 10 mM MgCl.sub.2, pH 9.5. After 45 minutes at room temperature, the absorbance of each well was measured at 410 nm on a Dynatech MR700 plate reader using diluent control wells as blanks. Tables 6 and 7 show the results for the rabbit control serum and EDC-conjugates.

Detailed Description Text (196):

The results in Table 6 show that the positive control serum, as expected, bound to LPS-coated wells in a specific manner. These data validate the ELISA design as being capable of detecting LPS binding antibodies.

Detailed Description Text (213):

In the previous two examples, cross-linkers were present in molar excess over IgG and were mixed simultaneously with both antibody and antibiotic. In this example, IgG was first modified with the cross-linker, the cross-linker removed, and then PMB added to the coupling reaction. In this way, the binding activity of PMB might be improved and the non-specific binding of the IgG reduced. In order to have an amine to amine coupling reagent that was more water soluble, BS.sup.3 (Pierce), a water soluble analogue of DSS was employed. The example involved: (a) two-step conjugation of IgG-PMB with EDC; (b) two step conjugation of IgG-PMB with BS.sup.3 ; and (c) ELISA of conjugate binding to LPS.

Detailed Description Text (239):

a) Periodate oxidation of IgG in pH 4.0 sodium acetate buffer was achieved by dissolving 5 mg human IgG in 1 ml of water and mixing this solution with 200 .mu.l of sodium acetate pH 4.0 (0.3 g sodium acetate and 960 .mu.l glacial acetic acid in 100 ml H.sub.2 O) and 200 .mu.l of 0.2 M NaIO.sub.4. [Modification of J. W. Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, New York, p. 84 (1986).] After 15 minutes at room temperature in the dark, the periodate solution was removed by gel filtration on a P-10 column in 50 mM Na.sub.2 CO.sub.3, pH 9.5.

Detailed Description Text (241):

Inspection of the LPS binding activity (not shown) revealed that the prepared conjugate was inactive. These results suggest that the periodate-oxidation of IgG, without the use of a cross-linker, is an ineffective means of covalent conjugation of antibiotics to antibody.

Detailed Description Text (244):

A significant concern with either one-step or multi-step schemes for conjugating antibiotics to antibodies is whether the conjugation scheme reduces or inactivates antibiotic function. In order to determine the best cross-linker concentration for derivatization of PMB in a multi-step conjugation scheme, the effect of the concentration of cross-linker on antibiotic activity was determined (see discussion of Mode IA above). The example involved: (a) modification of PMB with SPDP and the separation of free cross-linker; and (b) assay of derivatized PMB antibacterial activity.

Detailed Description Text (273):

Inhibition of Specific Binding of Antibiotic to LPS by Free Antibiotic

Detailed Description Text (274):

In order to determine that the antibody-antibiotic conjugate binding observed in FIG. 5 is specific, free antibiotic was used to block conjugate binding (see Mode III discussion, above). This example involved: (a) mixing of the antibiotic with free antibiotic; and (b) assaying the degree of conjugate binding to LPS in the presence of different concentrations of free antibiotic.

Detailed Description Text (275):

a) Mixing of antibiotic with free antibiotic was performed by adding an equal volume of a 1:125 dilution (32 .mu.g/ml) of the SPDP IgG-PMB conjugate in PBS-Tween 20 (0.05%) containing 1 mg/ml BSA with polymyxin at 0-20 .mu.g/ml in the same buffer. Two hundred (200) .mu.l of this mixture containing 0-2 .mu.g of PMB and 3.2 .mu.g of conjugate was then assayed for binding activity.

Detailed Description Text (277):

The results are shown in FIG. 7 and demonstrate that free polymyxin competitively inhibits IgG-PMB binding to LPS. Clearly, the antibiotic is binding specifically to LPS (i.e., via the conjugated PMB moieties).

Detailed Description Text (278):

Inspection of the inhibition curve gives some indication of the extent of active PMB conjugation, in that a 16 .mu.g/ml solution of antibody (1.1.times.10.sup.-7 M) is 50% inhibited in its binding to LPS by a concentration of 40 ng/ml PMB (2.6.times.10.sup.-8 M). If one molecule of PMB was present on each PMB (making the PMB concentration on IgG

equal to 1.1.times.10.sup.-7) one would expect that an equimolar concentration of free PMB would inhibit binding by 50%. Since it requires one fourth the concentration of free PMB to inhibit this antibioditic, one may conclude that there is at least one PMB molecule per four IgG molecules. In fact, since SPDP-modified PMB has a four-fold lower antibiotic activity than free PMB, the actual degree of IgG conjugation with PMB is probably at least four-fold higher than that calculated above (i.e., there is probably at least one PMB conjugated to each IgG molecule).

Detailed Description Text (282):

a) Periodate oxidation of IgG in phosphate buffer was achieved by dissolving 10 mg of human IgG in 1 ml of 50 mM NaPO.sub.4, pH 7.2 and adding 0.011 g sodium metaperiodate (final concentration 50 mM). After 30 minutes at room temperature, the periodate was removed by gel filtration on a 10 ml P-10 gel filtration column equilibrated in 50 mM NaPO.sub.4, pH 7.2. The peak fractions containing antibody were pooled and concentrated to 1.5 ml.

Detailed Description Text (343):

One of the functions of IgG is to opsonize and facilitate clearance of organisms, toxins, antigens, etc. by phagocytic cells. In order to determine whether this property of IgG, which is facilitated by the Fc region of the native molecule, remains intact in IgG conjugates that have been prepared with SPDP or periodate, the binding of IgG-PMB to human monocyte/macrophage cells was examined in a competition assay. This assay is similar to that employed to examine the Fc receptor binding activity of hybrid recombinant antibody fragments fused to cell surface viral receptors. [D. J. Capon et al., Nature 337:525-531 (1989); A. Traunecker et al., Nature, 339:68-70 (1989).] The example involved: (a) preparation of a control conjugate of PMB to human albumin (a non-Fc receptor binding human protein-PMB conjugate); and (b) assay of IgG-PMB conjugate binding to Fc receptors of the human U937 monocyte/macrophage cell line.

Detailed Description Text (348):

For the competition experiment, a constant quantity of .sup.125 I-IgG (1.times.10.sup.-8 M) was incubated with 2.times.10.sup.5 U937 cells in 0.5 ml of PBS containing 2 mg/ml BSA, 0.1% sodium azide and varying concentrations of the unlabelled competitor proteins: human IgG, IgG-PMB (SPDP), IgG-PMB (periodate), and human albumin-PMB from (a) above. The cells were incubated, washed, and the amount of bound radioactive .sup.125 I-IgG was quantitated as described above. In the absence of any of the human competitor proteins, 12,029 cpm of labelled IgG was bound to the cells. The results of the competitor assay are plotted in FIG. 10. It is clear that human IgG and both IgG-PMB conjugates have similar binding properties to the U937 cells in that all three compete comparably well at 10.sup.-8 M and 10.sup.-6 M. This result shows that the modification of the IgG with SPDP and PMB or by periodate oxidation of the carbohydrate side chains does not impair the ability of IgG to bind to Fc receptors. This suggests that the conjugates can facilitate Fc receptor-mediated opsonization of antigen/organisms by phagocytic cells. As expected, the human albumin-PMB exhibited no competitive binding activity at concentrations up to 10.sup.-6 (data not shown) and is therefore unable to facilitate opsonization.

Detailed Description Text (350):

Preparation of an Antibody-Antibiotic Conjugate with Activity Against Gram-Positive Bacteria: IgG-Bacitracin

Detailed Description Text (361):

Treatment of Persons Susceptible to Gram-Negative Sepsis And Endotoxemia with an Antibody-Antibiotic Conjugate

Detailed Description Text (364):

(b) Assay of endotoxin in core-antigen specific IgG and IgM levels is performed by ELISA. Plasma or sera are diluted and the level of binding of different sample dilutions to purified E. coli J5 endotoxin and Salmonella minnesota R595 endotoxin are quantitated and compared with known standards of purified anti-endotoxin antibodies. [B. J. Stoll et al., Serodiagnosis and Immunotherapy 1:21-31 (1987); and M. Pollack et al., J. Clin. Invest. 72:1874-1881 (1983).]

Detailed Description Text (367):

Because the IgG-PMB conjugates of the present invention comprise a population of antibody molecules all of which are capable of binding to endotoxin, much less IgG-PMB conjugate is required than total IgG to restore or increase levels antigen-specific antibody. A single intravenous dose consisting of 1-20 mg of IgG-PMB conjugate per kg of body weight is administered to restore endotoxin-specific antibody levels to

.gtoreq.100% of normal levels.

Detailed Description Text (369):
Treatment of Persons Susceptible to Gram-Negative Sepsis, Endotoxemia, and Gram-Positive Sepsis with a Cocktail of Antibody-Antibiotic Conjugates

Detailed Description Text (370):
Since there is a causal relationship between a person's humoral status and their susceptibility to infection, there is also a need to restore antibody levels against gram-positive organisms as well as the levels against gram-negative organisms and endotoxin. This is achieved by administration of a cocktail of antibody-antibiotic conjugates with activity against both classes of bacteria as well as endotoxin. The example involves: (a) identification of persons at risk of infection; and (b) administration of a cocktail of antibody-antibiotic conjugates and, if necessary, total pooled human immunoglobulin to restore antigen-specific and total immunoglobulin levels.

Detailed Description Text (372):
b) Administration of a cocktail of antibody-antibiotic conjugates and, if necessary, total pooled human immunoglobulin to restore antigen-specific and total immunoglobulin levels is carried out by injecting a single intravenous dose of IgG-PMB (1-20 mg/kg) and a single intravenous dose of IgG-bacitracin conjugate (1-20 mg/kg) to increase the levels of gram-negative and gram-positive-reactive antibodies, respectively. If total immunoglobulin levels are also .ltoreq.80% of normal, a 3% solution of intravenously injectable immunoglobulin (available from Sandoz Forschungsinstitut, Vienna, Austria; Hyland Therapeutics, Duarte, Calif.; or Cutter Laboratories, Berkeley, Calif.) is administered twice daily until immunoglobulin levels rise to within 10% of normal levels.

Detailed Description Text (391):
In order to detect bound antibodies, the wells were incubated with 100 .mu.l of a 1:500 dilution of goat anti-human IgG-alkaline phosphatase labeled antibody (Sigma) and incubated for 1 hour at 37.degree. C. After removing the secondary antibody solutions, the wells were washed 4 times with PBS-Tween-20. Substrate [p-nitrophenylphosphate (Sigma)] at 1 mg/ml in 50 mM Na.sub.2 CO.sub.3, and 10 mM MgCl.sub.2 was added to each well. The color developed after 15-20 minutes of incubation at room temperature was measured at 410 nm using a Dynatech MR700 microplate reader.

Detailed Description Text (395):
This example describes an experiment to determine if anti-PMB antibodies are elicited in rabbits by conjugate administration. Two rabbits were each given 3 mg of PMB-HiG conjugate intravenously on day 0. These rabbits received additional injections (boosts) at 2 weeks, 4 weeks and 7 weeks. As a control, 2 rabbits each received 3 mg of HiG alone at the same scheduled day and time as with the experimental group. All rabbits were bled every two weeks after receiving either conjugate or IgG alone. Sera were collected and stored at -70.degree. C. until tested for anti-PMB antibodies.

Detailed Description Text (396):
In order to detect anti-PMB antibodies in rabbit serum, a simple indirect binding assay was developed. Each well of a 96-well microtiter plate (Coming) was coated with 100 .mu.l of a 200 .mu.g/ml solution of PMB (Sigma) in endotoxin-free PBS. Control wells were coated with PBS only (no PMB). After an overnight incubation at 4.degree. C., the coating solutions were removed and all wells were washed 3 times with endotoxin-free PBS-Tween-20. The remaining antigen binding sites were blocked by the addition of PBS containing 10 mg/ml BSA (Sigma, tissue culture grade) for 1 hour at 37.degree. C. The blocking solution was removed and test rabbit serum samples diluted in 2% normal rabbit serum at dilutions of 1:10, 1:100, 1:1000 and 1:10,000 were added. A positive control antiserum (chicken anti-PMB immunoglobulin, Ophidian Pharmaceuticals Inc., Madison, Wis.) was also diluted as for the test rabbit serum samples. Samples were incubated in duplicate at 37.degree. C. for 1 hour. Following this incubation, the plates were washed three times with PBS-Tween-20.

Detailed Description Text (397):
In order to detect bound antibodies, the wells incubated with rabbit serum were incubated with 100 .mu.l of a 1:500 dilution of goat anti-rabbit IgG-alkaline phosphatase labeled antibody (Sigma) and the wells incubated with chicken antibody were incubated with 100 .mu.l of 1:500 dilution of goat anti-chicken IgG (whole molecule)-alkaline phosphatase conjugate (Sigma) for 1 hour at 37.degree. C. After removing the secondary antibody solutions, the wells were washed 4 times with

PBS-Tween-20 and p-nitrophenylphosphate (Sigma) at 1 mg/ml in 50 mM Na.sub.2 CO.sub.3, 10 mM MgCl.sub.2 was added to each well. The color developed after 15-20 minutes of incubation at room temperature was measured at 410 nm using a Dynatech MR700 microplate reader.

Detailed Description Text (398):

The results in Table 28 show that the positive control antibody, as expected, bound to PMB. This validates that the design of ELISA is capable of detecting PMB-binding antibodies. The results in Table 29 (shown as A.sub.410 readings of duplicate samples) indicate that none of the rabbit serum samples bound to PMB, indicating the absence of anti-PMB antibodies. These results demonstrate that PMB is not immunogenic, even on an heterologous protein carrier with repeated injections when given intravenously.

Detailed Description Text (425):

a) Periodate oxidation of IgG in phosphate buffer was achieved by dissolving 25 mg rat IgG (Sigma) in 1 ml of 50 mM NaPO.sub.4, pH 7.2 buffer and adding 10.7 mg of sodium metaperiodate (final concentration 50 mM). After 30 minutes of incubation at room temperature with gentle vortexing every 5 minutes, the periodate was removed by gel filtration on a 15 ml Swift desalting column (Pierce) equilibrated with 50 mM NaPO.sub.4, pH 7.2 buffer. The peak fractions containing highest amount of antibody as monitored by A.sub.280 absorbance were pooled.

Detailed Description Text (430):

Opsonic IgG class antibodies mediate an important immune effector function by enhancing the phagocytic clearance of extracellular bacteria [Raff, et al., J. Infect. Dis. 163:346-354 (1991).] In this way, opsonic IgG plays a critical role in host defense mechanisms against bacterial pathogens. [Rozenberg-Arska, et al. J. Med. Microbiol. 22:143-149 (1991).] The purpose of this example was to investigate whether the IgG component of IgG-PMB conjugates retains this important effector function. This was done by assessing whether the pre-treatment of E. coli organisms with IgG-PMB conjugate potentiates phagocytic uptake (opsonophagocytosis) by the human monocytic cell line U937. Opsonophagocytosis assays provide a useful means by which the potential therapeutic efficacy of immunoglobulin preparations, used for the treatment of bacterial infection, can be assessed. [Hill, et al. Am. J. Med. 61-66 (1984).] This example involved (a) Assay for opsonophagocytic activity of IgG-PMB conjugate, and (b) Determination of the minimum effective concentration of IgG-PMB conjugate.

Detailed Description Text (438):

The five suspensions were opsonized by incubation at 37.degree. C. for 60 min. with periodic mixing. Following opsonization, the suspensions were centrifuged as above, and the resulting pellets were each resuspended in 0.5 ml of RPMI 1640 medium which was supplemented with 10% FCS (this will be referred to as "medium" for the remainder of this example). Into each of 5 separate polypropylene culture tubes (S/P) was placed 1.0 ml of a U937 cell suspension, which was prepared in medium, and contained 1.times.10.sup.6 U937 cells/ml. To each tube, 0.1 ml of one of the opsonized E. coli suspensions prepared above was also added. A sixth control group was also prepared which contained 1.0 ml of the U937 cell suspension and 0.1 ml of PBS (PBS control). At this point, each tube contained 1.times.10.sup.6 U937 cells, and 2.times.10.sup.7 E. coli organisms, thus providing an E. coli to U937 cell ratio of 20:1. The 6 tubes were then incubated at 37.degree. C. for 60 min. with constant shaking, in order to allow phagocytosis to occur. Following incubation, the tubes were placed on ice for several minutes to prevent further phagocytosis. The 6 tubes were then centrifuged for 10 min. at 500.times.g at 4.degree. C. The resulting pellets were washed three times (centrifuging as in the previous step) with chilled PBS, to remove extracellular E. coli organisms. The final pellets were each resuspended in 0.2 ml of chilled PBS, and smears were prepared by applying 40 .mu.l volumes of the suspensions to glass microscope slides. The smears were allowed to air-dry, and were then fixed by immersion in 100% methanol for 5 sec. and again allowed to air-dry.

Detailed Description Text (545):

E. coli 0111:B4 lipopolysaccharide (LPS) was obtained from Sigma and was dissolved at 0.02 mg/ml in PBS plus 0.005% thymerosol. E. coli HB101 was diluted to 10,000,000 CFU/ml in PBS. 100 .mu.l aliquots of LPS solution, E. coli HB101 suspension or PBS were added to wells of Falcon Pro-Bind 96 well microtiter plates. The plates were incubated for 18 hours at 2-8.degree. C. The wells were washed 3 times with PBS. 100 .mu.l of PBS plus 5 mg/ml BSA (Sigma Chemical Co.) was added to each well of the plates and the plates were incubated for 2.0 hours at room temperature. The plates were decanted and 100 .mu.l of sample (e.g. conjugate, antibody, etc.) was added per well and the plates were incubated at ambient temperature for 2.0 hours. The wells were washed 6 times with

BBS (0.125 sodium borate, 1.0 M NaCl, pH 8.3) plus 0.5% Tween 20, 3 times with 50 mM sodium carbonate, pH 9.5. Three Sigma 104 phosphatase substrate tablets were dissolved in 15 ml of 50 mM sodium carbonate buffer plus 10 mM MgCl₂ and added at 100 ml per well. After approximately 20 minutes at ambient temperature, the absorbance at 410 nm of each well was determined.

Detailed Description Text (646):

Fluorescence polarization was utilized to determine if Immunoadapter.TM. compounds containing the hapten FL and the LPS binding ligand PMB bind both anti-fluorescein antibody and LPS respectively. Fluorescence polarization was first described in 1926 (Perrin, J. Phys. Rad 1:390:401) and has since been used to study many facets of molecular interactions. When fluorescent molecules are excited with plane polarized light, they emit in the same polarized plane provided that the molecules remain stationary throughout the excited state. However, if the excited molecule rotates or tumbles during the excited state, then the emitted light is in a different plane from the excitation light. When vertically polarized light is used to excite molecules, the emission light intensity can be monitored in both vertical and horizontal planes to detect movement of the molecule during excitation. If fluorescent molecules are very large, they move very little during excitation and the light from excitation to emission remains highly polarized. If fluorescent molecules are small, they rotate or tumble faster and the resulting emitted light is depolarized relative to the incident exciting light. In practice, small molecules (MW<1000) with fluorescent lifetimes of around 10 nanoseconds lose all their fluorescence polarization when they tumble and rotate freely. However, these molecules retain polarization when they are bound to larger molecules. A schematic diagram of a basic fluorescence polarization instrument is shown in FIG. 15.

Detailed Description Text (648):

The example involved a) Fluorescence polarization studies and b) Binding of anti-fluorescein antibody to fluoresceinated PMB-coated bacteria

Detailed Description Text (654):

An experiment was performed on an Ophidian fluorescence polarimeter (re-engineered fluorometer) using a more active fluoresceinated cysteine polymyxin B (PMB-Cys-Mal-FL conjugate). Millipolarization data was hand calculated using the above equations as described for the fully automated instrument. Total intensity is not represented in the following tables. Table 48 illustrates the reactivity of PMB-Cys-FL when reacted with mouse monoclonal anti-fluorescein antibody (#4-4-20) obtained from Edward M. Voss at the University of Illinois Urbana.

Detailed Description Text (655):

From Table 48 it is clear that anti-fluorescein antibody strongly polarizes PMB-Cys-Mal-FL and while not represented here, quenches 95% of the intensity signal. This demonstrates that PMB-Cys-FL can bind anti-fluorescein antibody.

Detailed Description Text (658):

II. Binding of Anti-fluorescein Antibody to Fluoresceinated PMB-coated Bacteria Results in Quenching of Fluorescence

Detailed Description Text (661):

The bacteria were diluted 10 fold with buffer and fluorescence was measured in a Sequoia-Turner model 450 Fluorometer using NB490 and SC515 filters with the gain at 5. Mouse monoclonal anti-fluorescein antibody 4-4-20 was added with mixing to the bacterial suspensions. Table 50 summarizes the experimental results.

Detailed Description Text (663):

When anti-fluorescein antibody binds to fluorescein, the fluorescence is reduced or quenched about 95%. If the fluorescein on fluoresceinated PMB coated E. coli is available on the exterior surface of the bacteria and anti-fluorescein antibody can bind then a reduction of signal is expected. If fluorescein is buried under the surface of the bacteria and anti-fluorescein antibody can not access it, no quenching of the fluorescence by anti-fluorescein antibody would be possible. The fluorescence of the Fluoresceinated PMB labeled E. coli is quenched to about 1/3 by 1 .mu.g/ml and higher concentrations of anti-fluorescein antibody. Thus, about 2/3 of the fluorescein on the coated E. coli is freely available to interact and bind to anti-fluorescein antibody. This demonstrates that PMB-Cys-Mal-FL can simultaneously bind both anti-fluorescein antibody and the bacterial surface.

Detailed Description Text (666):

It is desired that the PMB-Cys-Biotin conjugate (Example 39) bind to avidin or anti-biotin through the biotin hapten, in a manner that allows PMB to simultaneously bind its target. An ELISA assay was designed, to assess whether PMB-Cys-Biotin can simultaneously bind avidin and anti-PMB antibody. This example involved testing the PMB-Cys-Biotin conjugate in a capture ELISA.

Detailed Description Text (668):

The blocking solution was decanted, and duplicate samples of 150 .mu.l of either PMB-Cys-Biotin or PMB-Cys-Fl added to the first well of a dilution series. The initial testing concentration of the premixed conjugate was 10 ng/ml in blocking buffer (see above), followed by 5 fold dilutions in blocking buffer. This was accomplished by serially transferring 30 .mu.l aliquots to 120 .mu.l buffer, mixing, and repeating the dilution into a fresh well. After the final dilution, 30 .mu.l was removed from the well such that all wells contained 120 .mu.l final volume. A total of 2 such dilutions were performed. The plates were incubated 1.5 hr at 37.degree. C. Following this incubation, the serially diluted samples were decanted, and the wells were washed six times using PBST (Phosphate-Buffered Saline with Tween). To each well, 100 .mu.l of 1/1000 diluted chicken anti-PMB antibody or chicken preimmune, diluted in blocking buffer was added, and the plate incubated 1 hr at 37.degree. C. The antibody solutions were decanted and the plates were washed as described above. To each well, 100 .mu.l of 1/1000 diluted rabbit anti-chicken IgG alkaline phosphatase (Sigma) diluted in blocking buffer was added, and the plate incubated 1 hr at 37.degree. C. The antibody solutions were decanted and the plates were washed as described above, substituting 50 mM Na.sub.2 CO.sub.3, pH 9.5 for the PBST in the final wash. The plates were developed by the addition of 100 .mu.l of a solution containing 1 mg/ml para-nitro phenyl phosphate (Sigma, St. Louis, Mo.) dissolved in 50 mM Na.sub.2 CO.sub.3, 10 mM MgCl.sub.2, pH 9.5 to each well, and incubating the plates at room temperature in the dark overnight. The absorbency of each well was measured at 410 nm using a Dynatech MR 700 plate reader. The results are summarized in Table 51, and represent mean reactivities of duplicate wells. Only the ELISA readings from the maximally diluted samples in each dilution series are shown.

Detailed Description Text (669):

The ELISA clearly demonstrates that the PMB-Cys-Biotin conjugate can simultaneously bind avidin and anti-PMB antibody. This indicates that the Cys linker separates the hapten and the ligand in a manner that allows binding to both sites, as was demonstrated for PMB-Cys-Mal-FL in Example 41.

Detailed Description Text (672):

The MIC determinations shown in Example 40 demonstrated that the PMB-Cys-Biotin compound binds bacteria. For an Immuno adaptor.TM. compound to target hapten specific antibodies to the bacteria, binding must be to the bacterial surface, and not an internal target (e.g., the inner cytoplasmic membrane). To determine if PMB-Cys-Biotin stably binds to an accessible surface exposed target, bead agglutination assays were performed. The example involved a) generation of avidin coated polystyrene beads b) binding of PMB-Cys-Biotin to avidin beads and c) bacterial agglutination assays.

Detailed Description Text (687):

Immuno adaptor.TM. Compound Mediated Opsonization of J5 Cells

Detailed Description Text (688):

The agglutination studies of Example 43 demonstrated binding of PMB-Cys-Biotin to bacterial cells. A bacterial binding assay (BBA) was performed, to determine if this conjugate could also opsonize bacteria by targeting antibodies to bacteria. The J5 bacterial strain was selected for this study, since this strain had the strongest positive agglutination signal in example 43. A PMB-Cys-Biotin/extravidin premixed conjugate was utilized, since the increased avidity of this tetrameric complex may increase bacterial binding relative to monomeric PMB-Cys-Biotin. The example involved performing a BBA (Bacterial Binding Assay) using a ELISA Format.

Detailed Description Text (691):

The blocking solution was decanted, and duplicate samples of 150 .mu.l of each premixed conjugate added to the first well of a dilution series. The initial testing concentration of the premixed conjugate was 1 .mu.g/ml in blocking buffer (see above), followed by 5 fold dilutions into this buffer. This was accomplished by serially transferring 30 .mu.l aliquots to 120 .mu.l buffer, mixing, and repeating the dilution into a fresh well. After the final dilution, 30 .mu.l was removed from the well such that all wells contained 120 .mu.l final volume. A total of 2 such dilutions were performed (3 wells total). The plates were incubated 40 min at 37.degree. C. Following

this incubation, the serially diluted samples were decanted, and the wells were washed six times using PBST. To each well, 100 .mu.l of 1/5000 diluted rabbit anti-avidin antibody (Sigma) diluted in blocking buffer was added, and the plate incubated 1 hr at 37.degree. C. The conjugate solutions were decanted and the plates were washed as described above. To each well, 100 .mu.l of 1/1000 diluted goat anti-rabbit IgG alkaline phosphatase (Sigma) diluted in blocking buffer was added, and the plate incubated 1 hr at 37.degree. C. The conjugate solutions were decanted and the plates were washed as described above, substituting 50 mM Na.sub.2 CO.sub.3, pH 9.5 for the PBST in the final wash. The plates were developed by the addition of 100 .mu.l of a solution containing 1 mg/ml para-nitro phenyl phosphate (Sigma, St. Louis, Mo.) dissolved in 50 mM Na.sub.2 CO.sub.3, 10 mM MgCl.sub.2, pH 9.5 to each well, and incubating the plates at room temperature in the dark overnight. The absorbency of each well was measured at 410 nm using a Dynatech MR 700 plate reader. The results are summarized in Table 54, and represent mean reactivities of duplicate wells.

Detailed Description Text (692):

Although some background binding of the control PMB-Cys-Mal-FL premixed conjugate is observed, much stronger binding was observed (even at 40 ng/ml concentration) in J5 wells (but not PBS wells) incubated with PMB-Cys-Biotin but not PMB-Cys-Mal-FL (control) premixed conjugates. The low signal in the PMB-Cys-Mal-FL control lane indicates that extravidin needs linkage to PMB (through biotin in PMB-Cys-Biotin) for effective J5 bacterial binding. The detected background may be due to non-specific 'stickiness' of PMB containing conjugates, which may be elevated in the tetrameric PMB-Cys-Biotin/extravidin conjugate. These results clearly show that the PMB-Cys-Biotin premixed conjugate stably binds J5 cells (i.e., is not removed by washing 6 times with PBST) and can subsequently recruit anti-avidin. This demonstrates that the PMB-Cys-Biotin premixed conjugate can opsonize J5 cells by recruiting anti-avidin.

Detailed Description Text (694):

Immunoadaptor.TM. Compound Mediated Opsonization of a Pathogenic Bacteria

Detailed Description Text (695):

In Example 44, it was demonstrated that the PMB-Cys-Biotin/Extravidin premixed conjugate could direct anti-avidin opsonization of J5 bacteria. To determine if bacterial binding and opsonization can also be demonstrated to a encapsulated pathogenic bacteria, a modified bacteria binding assay was developed.

Detailed Description Text (707):

Tubes 1-7 were incubated 15 min at room temp, cells pelleted by centrifugation 5 min at 500 rpm in microcentrifuge, then resuspended in 1 ml Extravidin solution (2 .mu.g/ml in PBS). The samples were incubated 30 min at room temp, then all 10 samples were centrifuged as described above. The pellets were washed X with PBS (resuspended pellets in 1 ml PBS and recentrifuged for each wash). The pellets were resuspended in 1 ml PBS, and 100 .mu.l of a 1/10 dilution (2.times.10.sup.6 cells/ml) or a 1/100 dilution (2.times.10.sup.5 cells/ml) were pipetted into duplicate wells of a 96-well microtiter plates (Falcon, Pro-Bind Assay Plates). The wells were coated 2 hrs at room temperature. The bacterial suspensions were decanted, and all wells were washed three times using PBS. In order to block non-specific binding sites, 100 .mu.l of 1% gelatin (Sigma, St. Louis, Mo.) in PBS (blocking solution) was then added to each well, and the plates were incubated for 1 hr. at 37.degree. C. The blocking solution was decanted, and 100 .mu.l of 1/5000 diluted rabbit anti-avidin antibody (Sigma) in blocking buffer with 0.5% Tween 20 was added to each well, and the plate stored overnight at 4 degrees, then incubated 1 hr at 37.degree. C. The antibody solutions were decanted and the plates were washed as described above. To each well, 100 .mu.l of 1/1000 diluted goat anti-rabbit IgG alkaline phosphatase (Sigma) in blocking buffer with 0.5% Tween 20 was added, and the plate incubated 1 hr at 37.degree. C. The antibody solutions were decanted and the plates were washed as described above, substituting 50 mM Na.sub.2 CO.sub.3, pH 9.5 for the PBST in the final wash. The plates were developed by the addition of 100 .mu.l of a solution containing 1 mg/ml para-nitro phenyl phosphate (Sigma, St. Louis, Mo.) dissolved in 50 mM Na.sub.2 CO.sub.3, 10 mM MgCl.sub.2, pH 9.5 to each well, and incubating the plates at room temperature in the dark overnight. The absorbency of each well was measured at 410 nm using a Dynatech MR 700 plate reader. The results are summarized in Table 55, and represent mean reactivities of duplicate wells.

Detailed Description Text (710):

2) Extravidin binding to prebound PMB-Cys-Biotin or single step PMB-Cys-Biotin/extravidin premixed conjugate binding to bacteria is stable (the bacteria were coated 3 hr at room temperature, washed, and blocked 1 hr before

anti-avidin antibody was added.

Detailed Description Text (711):

3) Bacterially bound PMB-Cys-Biotin/extravidin complexes, assembled using premix or chase format, can recruit anti-avidin antibody to H16 bacteria. The relative signal is clearly higher than the control PMB-Cys-Mal-FL chase or premix with extravidin. This clearly indicates PMB containing Immunoadapter.TM. compound mediated anti-avidin opsonization of the pathogenic encapsulated H16 strain.

Detailed Description Text (712):

4) Single step C90-99-Cys-Biotin/extravidin premixed conjugate binding to bacteria is stable (the bacteria were coated 3 hr at room temperature, washed, and blocked 1 hr before anti-avidin antibody was added).

Detailed Description Text (713):

5) Bacterially bound C90-99-Cys-Biotin/extravidin complexes, assembled using premix (but not chase format), can recruit anti-avidin antibody to H16 bacteria. The relative signal is clearly higher than the control PMB-Cys-Mal-FL chase or premix with extravidin. This clearly indicates C90-99 containing Immunoadapter.TM. compound mediated anti-avidin opsonization of the pathogenic encapsulated H16 strain.

Detailed Description Text (716):

Phagocytosis of PMB-Cy-Biotin/Extravidin Anti-Avidin Opsonized J5 cells: Chase Format

Detailed Description Text (717):

As outlined in Example 45, the chase format utilizing a PMB-Cys-Biotin/extravidin premixed conjugate with an anti-avidin antibody chase maximally opsonized H16 bacteria. This format was selected for initial determination if Immunoadapter.TM. compounds can be utilized to stimulate immune effector functions. The example involved I) development of a phagocytosis assay and II) assessment of phagocytosis of PMB-Cys-Biotin/extravidin/anti-avidin opsonized J5 bacteria relative to that of J5 cells opsonized with control reagents.

Detailed Description Text (720):

Opsonized bacteria were prepared as follows. A 5 ml 2XYT media bacterial culture was set, and bacteria grown at 37.degree. C. until mid-log phase (0.5-1.0 OD.sub.600). Cells were pelleted by centrifugation 5 min at 3000 rpm in Beckman GS-6 centrifuge, and bacteria resuspended (vortexing) to density of 1×10^9 cells/ml (1.0 OD.sub.600) in HBSS. Cells were diluted 1/10 in HBSS in 1.5 ml siliconized microcentrifuge tubes, vortexed, and test opsonins added. The samples were incubated 15-20 min at room temperature, then stored on ice until use. Samples were vortexed immediately before adding to macrophages. Vortexing is important to prevent bacterial clumping, which would result in macrophage adherent clumps of bacteria.

Detailed Description Text (721):

1-2.5.times. 10^6 J774 cells were diluted to 0.9 ml with HBSS in Falcon 2063 tubes, and 1.times. 10^6 opsonized bacteria (0.1 ml of opsonized bacteria from above) immediately added, the tube capped, and rotated (end over end) at 37 degrees for 20 minutes to allow phagocytosis of opsonized bacteria. Samples were centrifuged 5 min at 250 g in a Beckman GS-6 centrifuge at 4 degrees. This pellets macrophages and associated bacteria but not free bacteria. The supernatant was decanted (macrophages visible as white pellet), 2 ml HBSS added, and the macrophage pellet carefully resuspended with a P-1000 pipette. The sample was repelleted and rewashed a total of 3.times. The pellet was resuspended in 1 ml HBSS, underlayered with 1 ml 30% sucrose and recentrifuged as above for 8 minutes. The supernatant was carefully removed with a P-1000, and the pellet resuspended in 1 ml HBSS. Samples were maintained on ice throughout the experiment to limit killing of internalized bacteria.

Detailed Description Text (723):

II. Assessment of Phagocytosis of PMB-Cys-Biotin/Extravidin/Anti-Avidin Opsonized J5 Bacteria Relative to that of J5 Cells Opsonized With Control Reagents

Detailed Description Text (724):

Six opsonized bacterial samples were assayed for macrophage phagocytosis. The samples were prepared as outlined below.

Detailed Description Text (726):

A stock of 1/10 diluted, J5 absorbed anti-avidin antibody was prepared as follows. Approximately 1×10^9 J5 cells from an overnight TSA plate were added to 1 ml

HBSS in a 1.5 ml microfuge tube. The cells were pelleted by centrifugation 1 min at 12,000.times.g in microfuge, resuspended in 0.9 ml HBSS and 100 .mu.l anti-avidin (rabbit polyclonal serum; Sigma) added. The tube was rotated 90 min at 4 degrees, the bacteria pelleted as described above. The sample was filter sterilized and stored at 4 degrees.

Detailed Description Text (734):

The bacteria were opsonized, and the phagocytosis assay performed as described above. Samples 1-4 were tested in duplicate independent experiments. The results are shown in Tables 56 and 57 below. (Note that the following key applies: 1) From 100 macrophage examined, 2) From 100 .mu.l plating of a 10⁻³ dilution of macrophage in dH.sub.2 O)

Detailed Description Text (735):

The results demonstrate that opsonization with PMB-Cys-Biotin/Extravidin premixed conjugate followed by an anti-avidin chase targets cells for phagocytosis by the macrophage cell line; the control opsonization samples clearly demonstrate that this enhancement is antibody dependent. Therefore these results provides proof of principle for the use of Immunoadapter.TM. compounds to target antibody effector functions, using avidin to link Immunoadapter.TM. compounds as tetramers with anti-avidin in a chase format.

Detailed Description Text (737):

Phagocytosis of PMB-Cys-Biotin/Extravidin/Anti-Avidin Opsonized J5 cells: Premix and Chase Format

Detailed Description Text (738):

As demonstrated in Example 46, opsonization of J5 bacteria utilizing a PMB-Cys-Biotin/extravidin premixed conjugate with an anti-avidin chase stimulated phagocytosis. Phagocytosis utilizing a PMB-Cys-Biotin/extravidin/antiavidin premix was assessed, to determine if a premix of the components can also target immune effector functions (phagocytosis). The example involved assessment of phagocytosis of PMB-Cys-Biotin/extravidin/anti-avidin opsonized J5 bacteria in a premix or chase format, relative to that of J5 cells opsonized with control reagents.

Detailed Description Text (739):

The phagocytosis assay was performed exactly as described in Example 46, using, where relevant, the same reagents as Example 46. Six opsonized bacterial samples were assayed for macrophage phagocytosis. Samples not described in Example 46 were prepared as follows.

Detailed Description Text (745):

4) 2.5 .mu.l PMB-Cys-Biotin/extravidin premixed conjugate (Example 6; 0.5 .mu.g/ml PMB-Cys-Biotin final concentration), 2 min room temp, +1 .mu.l anti-avidin monoclonal antibody (1/1000 final dilution)

Detailed Description Text (748):

The bacteria were opsonized, and the phagocytosis assay performed as described in Example 46 above. The results are shown in Table 58 below. (Note that for the following table the following key applies: 1) From 100 macrophage examined and 2) From 100 .mu.l plating of a 10⁻³ dilution of macrophage in distilled H.sub.2 O)

Detailed Description Text (749):

The results demonstrate that opsonization with PMB-Cys-Biotin/Extravidin premixed conjugate in a premix format with monoclonal anti-avidin or in a chase format with monoclonal or polyclonal anti-avidin targets cells for phagocytosis by the J774 macrophage cell line; the control opsonization samples clearly demonstrate that the observed enhancement of phagocytosis is dependant on the presence of both premixed conjugate and antibody. These results provides proof of principle for the use of Immunoadapter.TM. compounds to target antibody effector functions, using premix or chase formats with monoclonal or polyclonal antibodies.

Detailed Description Text (751):

Phagocytosis of PMB-Cys-Mal-FL/Antibody Opsonized J5 cells: Chase and Premix Format

Detailed Description Text (752):

As demonstrated in Examples 46 and 47, opsonization of J5 bacteria utilizing a PMB-Cys-Biotin/extravidin premixed conjugate with an anti-avidin premix or chase stimulated phagocytosis. In Example 41, it was demonstrated that PMB-Cys-Mal-FL could recruit anti-fluorescein antibody to the bacterial surface. The phagocytosis assay was

utilized, with PMB-Cys-Mal-FL and anti-fluorescein antibody, to determine if enhanced phagocytosis is also observed in premix and chase formats when an antibody reactive to the Immunoadapter.TM. compound hapten (rather than extravidin) is utilized. The example involved assessment of phagocytosis of PMB-Cys-Mal-FL/anti-fluorescein antibody opsonized J5 bacteria in a premix or chase format, relative to that of J5 cells opsonized with PMB-Cys-Mal-FL or anti-fluorescein antibody alone.

Detailed Description Text (753):

The phagocytosis assay was performed exactly as described in examples 46 and 47, using the PMB-Cys-Biotin/extravidin premixed conjugate and anti-avidin reagents described in Example 46. A PMB-Cys-Mal-FL/anti-fluorescein premixed conjugate was prepared as follows. In a 500 . μ l microfuge tube, 8.4 . μ l PMB-Cys-Mal-FL (1.0 . μ g) and 10 . μ l anti-fluorescein antibody (affinity purified 4-4-20 monoclonal, stock at 2 mg/ml) were mixed and incubated overnight at 4 degrees. Binding of anti-fluorescein antibody to the FL hapten was confirmed by the visible quenching of fluorescein in the sample (observed immediately after adding antiserum).

Detailed Description Text (754):

Six opsonized bacterial samples were assayed for macrophage phagocytosis. To 1.times.10.^{sup}.8 J5 bacteria in HBSS in microfuge tubes (prepared as described above), the following test solutions (from above) were added:

Detailed Description Text (757):

3) 5 . μ l anti-fluorescein antibody (10 . μ g/ml final concentration).

Detailed Description Text (759):

5) 9.2 . μ l PMB-Cys-Mal-FL/anti-fluorescein antibody premixed conjugate (0.5 . μ g/ml PMB-Cys-Mal-FL, 10 . μ g/ml anti-fluorescein antibody)

Detailed Description Text (760):

6) 4.2 . μ l PMB-Cys-Mal-FL, 2 min room temp, 5 . μ l antibody (0.5 . μ g/ml PMB-Cys-Mal-FL, 10 . μ g/ml anti-fluorescein antibody)

Detailed Description Text (761):

The bacteria were opsonized, and the phagocytosis assay performed as described above. The results are shown in Table 59 below. (Note that: 1=From 100 macrophage examined and 2=From 100 . μ l plating of a 10⁻³ dilution of macrophage in dH.sub.2 O)

Detailed Description Text (762):

The results demonstrate that opsonization with PMB-Cys-Mal-FL in a premix or chase format with monoclonal anti-fluorescein antibody targets cells for phagocytosis by the J774 macrophage cell line; the control opsonization samples clearly demonstrate that the observed enhancement of phagocytosis is dependant on the presence of both premixed conjugate and antibody. These observations are duplicated in a second independent assay (Example 49). These results provides proof of principle for the use of Immunoadapter.TM. compounds to target antibody effector functions, using premix or chase formats with a monoclonal antibody directly reactive to the Immunoadapter.TM. compound hapten.

Detailed Description Text (764):

Phagocytosis of C90-99-Cys-Biotin/Extravidin/Anti-Avidin Opsonized J5 Cells

Detailed Description Text (765):

As demonstrated in Examples 46-48, opsonization of J5 bacteria utilizing PMB containing conjugates in premix or chase formats stimulated phagocytosis. PMB is a member of a class of molecules that bind the bacterial cell surface through interactions with LPS. Conjugates of other LPS binding molecules (e.g., C90-99; Grey and Haseman, 1994, Infect. Immun. 62, 2732; and C19g; Darveau et al., 1992, J. Clin. Invest. 90, 447-455) were made to either Fl (C19g-Mal-FL; described in Example 39) or biotin (C90-99-Cy-Biotin; described in Example 39). The phagocytosis assay was used to determine if enhanced phagocytosis is also observed in chase formats when Immunoadapter.TM. compounds utilizing these non-PMB targeting ligands are utilized. This will determine if the observed enhanced phagocytosis in Examples 46-48 is PMB specific or is a general feature of Immunoadapter.TM. compounds that utilize LPS binding molecules. The example involved assessment of phagocytosis utilizing Immunoadapter.TM. compounds containing various LPS binding peptides as ligand domain.

Detailed Description Text (767):

Two independent phagocytosis assays were conducted, using independently opsonized

bacterial samples. To 1.times.10.sup.8 J5 bacteria in HBSS in microfuge tubes (prepared as described above), the following test solutions (from above) were added:

Detailed Description Text (773):

5) 3.5 .mu.l anti-fluorescein antibody (7 mg/ml stock, 25 .mu.g/ml final concentration)

Detailed Description Text (775):

7) 4.2 .mu.l PMB-Cys-Mal-FL, 2 min room temp, 3.5 .mu.l anti-fluorescein antibody (0.5 .mu.g/ml PMB-Cys-Mal-FL, 25 .mu.g/ml anti-fluorescein) (fluorescein chase)

Detailed Description Text (776):

8) 4.2 .mu.l PMB-Cys-Mal-FL+3.5 .mu.l anti-fluorescein antibody (0.5 .mu.g/ml PMB-Cys-Mal-FL, 25 .mu.g/ml anti-fluorescein antibody), premix 10 min room temperature (fluorescein premix)

Detailed Description Text (777):

The bacteria were opsonized, and the phagocytosis assay performed as described above. The results are shown in Table 60 below. (Note that 1=From 100 macrophage examined)

Detailed Description Text (778):

Clear elevation of phagocytosis was observed in the Fl chase and Fl premix samples (relative to controls) and a 2 fold increase with C90-99 premixed conjugate+anti-avidin was observed, relative to the C90-99 premixed conjugate or anti-avidin controls. The assay was repeated, to determine if the observed enhancement of phagocytosis is repeatable. As well, a C19g-Mal-FL conjugate was tested for enhancement of phagocytosis when incubated with anti-fluorescein antibody in a chase format.

Detailed Description Text (785):

5) 2.0 .mu.l anti-fluorescein antibody (7 mg/ml stock, 14 .mu.g/ml final concentration)

Detailed Description Text (787):

7) 4.2 .mu.l PMB-Cys-FL, 2 min room temp, 2.0 .mu.l antibody (0.5 .mu.g/ml PMB-Cys-FL, 14 .mu.g/ml anti-fluorescein antibody) (fluorescein chase)

Detailed Description Text (789):

9) 3.2 .mu.l C19g-Mal-FL, 2 min room temp, 2.0 .mu.l antibody (0.5 .mu.g/ml C19g-Mal-FL, 14 .mu.g/ml anti-fluorescein antibody) (C19g chase)

Detailed Description Text (790):

The bacteria were opsonized, and the phagocytosis assay performed as described above. The results are shown in Table 61 below. (Note that 1=From 100 macrophage examined)

Detailed Description Text (791):

The assay demonstrates that the 2 fold enhancement of phagocytosis by C90-99 premixed conjugate+anti-avidin seen above is reproducible. This enhancement is considerably lower than that observed with the PMB-Cys-Mal-FL/anti-fluorescein antibody chase, which utilizes PMB as a targeting ligand. This is likely to be due to the reduced affinity of the C90-99 ligand for LPS relative to PMB. Indeed, this reduced affinity is reflected by reduced bacterial binding of the C90-99-Cys-Biotin conjugate relative to the PMB-Cys-Biotin conjugate (Example 45). C19g also has reduced affinity for LPS relative to PMB; it is probably that this reduced affinity prevents the C19g-Mal-FL conjugate from enhancing bacterial phagocytosis in the assay. These results provides proof of principle for the use of Immunoadapter.TM. compounds containing LPS binding ligands to target antibody effector functions; the affinity of the LPS binding ligand will be critical for maximal opsonization and subsequent phagocytosis.

Detailed Description Text (797):

Several pathogenic bacteria have fimbriae or pili located on their surface. The present invention contemplates molecules that bind to the receptors on these pili may be used for targeting antibodies to these bacteria. Type 1 pili contain receptors for .alpha.-linked mannosides. Sharon has shown that the .alpha.-linked aromatic mannosides bind to the receptors on type 1 pili up to 700 times more strongly than methyl .alpha.-mannosides. (See N. Firon et al., Infect. Immunity 55:472 (1987)). The examples described by Sharon, however, have aromatic groups which do not permit any conjugation to other molecules.

Detailed Description Text (816):

Monoclonal antibodies to this epitope block ligand binding to the cells and bioengineering through amino acid switching disrupts the affinity of cell receptor interaction. Again, as in the previous examples for the demonstration of complement activation and lysis of erythrocytes, the receptor binding peptide will be substituted for the Clq binding peptide. The phagocytosis of red blood cells will be observed.

Detailed Description Paragraph Table (2):

TABLE 2
Antibiotics That May Be Conjugated To Antibodies Type Examples Activity Spectrum

Penicillins.sup.1 penicillin G, antibacterial, inhibition antibacterial, gram- amoxicillin, nafcillin, of cell wall synthesis positive and gram- ampicillin, ticarcillin, negative carbenicillin, cloxacillin, penicillin V Cephalosporins.sup.2 cefoxitin, ceforanide antibacterial, inhibition antibacterial, gram- of cell wall synthesis positive and gram- negative Polymyxin polymyxin B, colistin antibacterial binds and antibacterial, inhibits cell wall primarily gram- synthesis negative Vancomycin.sup.3 vancomycin, antibacterial, binds to antibacterial, teicoplanin, ristocetin cell wall precursor, primarily gram- inhibits synthesis positive Biosurfactants.sup.4 circulin, EM49, surface-active antibacterial polypeptin, brexistin, cerexin, tridecephin, surfactin surfactin, subsporin, surface-active fungicidal mycosubtilisin, bacillomycin Other Peptide viomycin, not known antimycobacterial Antibiotics.sup.5 capreomycin (tuberculostatic) bacitracin, gramicidin, surface-active antibacterial gramicidin S, tyrocidine Amantadine.sup.6 amantadine blocks ion channel antiviral (Influenza A) Polyene macrolide.sup.7 amphotericin surface activity on antifungal membrane sterols Endotoxin binding tachyplesin.sup.8 surface active antibacterial proteins Limulus anti-LPS LPS-binding antiendotoxin factor.sup.9 LPS binding protein LPS-binding anti-endotoxin (human).sup.10 bactericidal LPS-binding anti-endotoxin permeability increasing protein.sup.11

Mandell and M. A. Sande in Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., (Gilman, Rall, Nies, and Taylor, eds.), Pergamon Press, New York, pp. 1065-1097 (1990). .sup.2 Id. .sup.3 M. A. Sande and G. L. Mandell in Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., (Gilman, Rall, Nies, and Taylor, eds.), Pergamon Press, New York, pp. 1117-1145 (1990). .sup.4 A. Fiechter, Trends in Biotech. 10:208-217 (1992). .sup.5 G. L. Mandell and M. A. Sande in Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., (Gilman, Rall, Nies, and Taylor, eds.), Pergamon Press, New York, pp. 1146-1164 (1990). .sup.6 R. G. Douglas in Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., (Gilman, Rall, Nies, and Taylor, eds.), Pergamon Press, New York, pp. 1182-1201 (1990). .sup.7 J. E. Bennett in Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., (Gilman, Rall, Nies, and Taylor, eds.), Pergamon Press, New York, pp. 1165-1181 (1990). .sup.8 T. Nakamura et al., J. Biol. Chem. 263:16709-16713 (1988). .sup.9 G. Alpert et al., J. Infect. Dis. 165:494-500 (1992). .sup.10 R. R. Schumann et al., Science 249:1429-1431 (1990). .sup.11 M. N. Marra et al., J. Immunol. 148:532-537 (1992).

Detailed Description Paragraph Table (29):

TABLE 28 Binding Of Chicken Antibodies To PMB
Absorbance At 410 nm Preimmune Egg Anti-PMB Egg Dilution Of Antibody Yolk Antibodies
1:10 0.149 1.741 1:100 0.083
Yolk Antibodies
1.732 1:1,000 0.026 1.700 1:10,000 0.015 0.686 1:100,000 0.006 0.100

Detailed Description Paragraph Table (36):

TABLE 35 U937 Cells Which Opsonization Treatment
Contained One Or More E. coli IgG-PMB @ MIC
(0.062 mg/ml) 65% IgG-PMB @ 2 x MIC (0.25 mg/ml) 56% IgG @ 0.062 mg/ml 0% IgG @ 0.25 mg/ml 3% PBS Control 0% U937 Cells Only (No E. coli) 0%

Detailed Description Paragraph Table (37):

TABLE 36 U937 Cells Which Opsonization Treatment
Contained One Or More E. coli IgG-PMB @ MIC
(0.062 mg/ml) 38% IgG-PMB @ 1/2 MIC (0.031 mg/ml) 41% IgG-PMB @ 1/4 MIC (0.0155 mg/ml) 14% IgG-PMB @ 1/8 MIC (7.75 .mu.g/ml) 10% Alb-PMB @ 0.062 mg/ml 2% Alb-PMB @ 0.031 mg/ml 0% Alb-PMB @ 0.0155 mg/ml 2% Alb-PMB @ 7.75 .mu.g/ml 5% IgG @ 0.062 mg/ml 2% PBS Control 0%

Detailed Description Paragraph Table (53):

TABLE 48 Reactivity of PMB-Cys-Mal-FL with
 anti-Fluorescein Antibody SAMPLE IV IH mP
 Phosphate buffer (no intensity) 3 1 474.2 PMB-Cys-Mal-FL 10 .sup.-7 M 845 740 32.5
 antibody 5 .times. 10.sup.-8 M (no intensity) 3 1 474.2 antibody 5 .times. 10-8 M & 61
 25 390.3 PMB-Cys-Mal-FL 10-7 M

Detailed Description Paragraph Table (55):

TABLE 50 Quenching of Fluorescence on
 PMB-Cys-Mal-FL Coated Bacteria FLUORESCENCE (AFU)
 anti-fluorescein antibody 16 E. coli + 2 .mu.g/ml anti-fluorescein antibody 15 E. coli
 + 22 .mu.g/ml anti-fluorescein antibody 15

Detailed Description Paragraph Table (56):

TABLE 51 PMB-Cys-Biotin binds avidin and
 anti-PMB antibody simultaneously PBS Test Conjugate Test antibody Avidin coated wells
 coated wells PMB-Cys-Biotin Anti-PMB 1.252 0.016
 PMB-Cys-Biotin Preimmune 0.014 0.019 PMB-Cys-FL Anti-PMB 0.033 0.016 PMB-Cys-FL
 Preimmune 0.010 0.020

Detailed Description Paragraph Table (59):

TABLE 54 PMB-Cys-Biotin Directs Anti-Avidin
 Mediated Opsonization of J5 Bacteria Test Conjugate 1 .mu.g/ml J5 coated wells PBS
 coated wells PMB-Cys-Biotin/extravidin 1.591
 0.95 premixed conjugate 200 ng/ml PMB-Cys-Biotin/extravidin 1.557 0.25 premixed
 conjugate 40 ng/ml PMB-Cys-Biotin/extravidin 1.449 0.043 premixed conjugate 1 .mu.g/ml
 PMB-Cys- Mal-FL/extravidin 0.387 0.186 premixed conjugate 200 ng/ml PMB-Cys-
 Mal-FL/extravidin 0.176 0.061 premixed conjugate 40 ng/ml PMB-Cys- Mal-FL/extravidin
 0.089 0.02 premixed conjugate

Detailed Description Paragraph Table (60):

TABLE 55 Anti-Avidin Antibody Opsonization
 [Coated H16 Bacteria] ELISA titer ELISA titer (2 .times. 10.sup.6 (2 .times. 10.sup.5
 Sample (conjugate) Format bacteria) bacteria) 1)
 1 .mu.g/ml Extravidin and 0.162 0.005 PMB-Cys-Mal-FL anti-avidin chase 2) 1 .mu.g/ml
 Extravidin and 1.389 0.776 PMB-Cys-Biotin anti-avidin chase 3) 100 ng/ml Extravidin and
 1.037 0.050 PMB-Cys-Biotin anti-avidin chase 4) 10 ng/ml Extravidin and 0.398 0.000
 PMB-Cys-Biotin anti-avidin chase 5) 1 .mu.g/ml Extravidin and 0.238 0.033
 C90-99-Cys-Biotin anti-avidin chase 6) 100 ng/ml Extravidin and 0.101 0.035
 C90-99-Cys-Biotin anti-avidin chase 7) 10 ng/ml Extravidin and 0.002 0.000
 C90-99-Cys-Biotin anti-avidin chase 8) 1 .mu.g/ml Premixed conjugate 1.455 1.426
 PMB-Cys-Biotin/ with anti-avidin chase Extravidin 9) 1 .mu.g/ml Premixed conjugate
 1.416 1.258 C90-99-Cys-Biotin/ with anti-avidin chase Extravidin 10) 1 .mu.g/ml
 Premixed conjugate 0.813 0.349 PMB-Cys-Mal-FL/ with anti-avidin chase Extravidin

Detailed Description Paragraph Table (61):

TABLE 56 Opsonization With
 PMB-Cys-Biotin/Extravidin in a Chase Format with Anti-Avidin Enhances Phagocytosis %
 Macrophage with Recovered bacterial Test Conjugate phagocytosed bacteria.sup.1
 colonies.sup.2 PMB-Cys-Biotin/extravidin 60 178
 (premixed) + anti-avidin PMB-Cys-Biotin/extravidin 23 67 premixed conjugate anti-avidin
 14 46 negative control 14 72

Detailed Description Paragraph Table (62):

TABLE 57 Opsonization with
 PMB-Cys-Biotin/Extravidin in a Chase Format With Anti-Avidin Enhances Phagocytosis
 Relative to PMB-Cys-Biotin or Extravidin Opsonized Cells % Macrophage with phagocytosed
 Recovered bacterial Test Conjugate bacteria.sup.1 colonies.sup.2
 anti-avidin PMB-Cys-Biotin/extravidin 16 72 premixed conjugate anti-avidin 24 80
 negative control 21 78 PMB-Cys-Biotin + anti-avidin 21 74 Extravidin + anti-avidin 22
 57

Detailed Description Paragraph Table (63):

TABLE 58 Opsonization With
 PMB-Cys-Biotin/Extravidin in a Premix or Chase Format with Anti-Avidin Enhances
 Phagocytosis Recovered % Macrophage with bacterial Test Conjugate phagocytosed

bacteria.sup.1 colonies.sup.2
 PMB-Cys-Biotin/extravidin 62 222 premixed conjugate + anti-avidin polyclonal
 PMB-Cys-Biotin/extravidin 25 54 premixed conjugate anti-avidin monoclonal 22 30
 negative control 23 76 PMB-Cys-Biotin/extravidin + 63 154 anti-avidin monoclonal
 (premix) PMB-Cys-Biotin/ 75 208 extravidin + anti-avidin monoclonal (chase)

Detailed Description Paragraph Table (64):

TABLE 59 Opsonization with
PMB-Cys-Biotin/Extravidin in a Premix or Chase Format with Anti-Avidin Enhances
Phagocytosis Recovered % Macrophage with bacterial Test Conjugate phagocytosed
 bacteria.sup.1 colonies.sup.2
 PMB-Cys-Biotin/extravidin 60 91 premixed conjugate + anti-avidin polyclonal Negative
 control 13 21 anti-fluorescein 14 6 PMB-Cys-Mal-FL 15 29
 PMB-Cys-Mal-FL/anti-fluorescein 29 52 antibody (premix) PMB-Cys-Mal-FL anti-fluorescein
 40 76 antibody (chase)

Detailed Description Paragraph Table (65):

TABLE 60 Opsonization with
C90-99-Cys-Biotin/Extravidin in a chase format with anti-avidin % macrophage with Test
Conjugate phagocytosed bacteria.sup.1
 Negative control 2 2) C90-99 premixed conjugate + anti-avidin 8 3) anti-avidin 3 4)
 C90-99 premixed conjugate 3 5) anti-fluorescein 4 6) PMB-Cys-Mal-FL 5 7) Fluorescein
 chase 14 8) Fluorescein premix 12

Detailed Description Paragraph Table (66):

TABLE 61 Opsonization with
C90-99-Cys-Biotin/Extravidin in a Chase Format with Anti-Avidin Enhances Phagocytosis %
macrophage with Test Conjugate phagocytosed bacteria.sup.1
 conjugate + anti-avidin 19 3) anti-avidin 4 4) C90-99 premixed conjugate 4 5)
 anti-fluorescein 13 6) PMB-Cys-Mal-FL 8 7) Fluorescein chase 43 8) C19g-Mal-FL 8 9)
 C19g chase 10

Other Reference Publication (5):

K.A. Schulman et al., "Cost-Effectiveness of HA-1A Monoclonal Antibody for
 Gram-Negative Sepsis: Economic Assessment of a New Therapeutic Agent," JAMA
 266:3466-3471 (1991).

Other Reference Publication (6):

C.J. Fisher et al., "Initial Evaluation of Human Monoclonal Anti-Lipid A Antibody
 (HA-1A) in Patients with Sepsis Syndrome," Clin. Care Med., 18:1311-1315 (1990).

Other Reference Publication (7):

C.H.J. Ford et al., "Antibody Mediated Targeting of Radioisotopes, Drugs and Toxins in
 Diagnosis and Treatment," Indian J. Pediatr., 57:29-46 (1990).

Other Reference Publication (26):

M. Pollack et al., "Enhanced Survival in Pseudomonas aeruginosa Septicemia Associated
 with High Levels of Circulating Antibody to Escherichia coli Endotoxin Core," J. Clin.
 Invest., 72:1874-1881 (1983).

Other Reference Publication (27):

S.H. Zinner and W.R. McCabe, "Effects of IgM and IgG Antibody in Patients with
 Bacteremia Due to Gram-negative Bacilli," J. Infect. Dis., 133:37-45 (1976).

Other Reference Publication (28):

B.J. Stoll et al., "Antibodies to Endotoxin Core Determinants in Normal Subjects and in
 Immune Globulins for Intravenous Use," Serodiagnosis and Immunotherapy 1:21-31 (1987).

Other Reference Publication (29):

W. Marget et al., "Lipid A Antibody Determinations Using ELISA on Patients at a
 Children's Hospital: A Preliminary Report," Infection 11:84-86 (1983).

Other Reference Publication (30):

C. Stoll et al., "Serum Antibodies Against Common Antigens of Bacterial
 Lipopoly-saccharides in Healthy Adults and in Patients with Multiple Myeloma,"
 Infection 13:115-119 (1985).

Other Reference Publication (37):
R.L. Greenman et al., "A Controlled Clinical Trial of E5 Murine Monoclonal IgM Antibody to Endotoxin in the Treatment of Gram-Negative Sepsis," JAMA 266:1087-1102 (1991).

Other Reference Publication (38):
M.A. Awad et al., "Plasma Endotoxin and Glycolipid Antibodies in Children with Meningitis," Acta Paediatrica, 81:560-561(1992).

Other Reference Publication (47):
J.W. Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, New York, p. 84 (1986).

Other Reference Publication (57):
Raff et al., "Comparison of Functional Activities Between IgG1 and IgM Class-Switched Human Monoclonal Antibodies Reactive with Group B Streptococci or Escherichia coli K1," J. Infect. Dis., 163:346-354 (1991).

Other Reference Publication (60):
Gemmell et al., "Potentiation of Opsonization and Phagocytosis of Streptococcus pyogenes following Growth in the Presence of Clindamycin," J. Clin. Invest., 67:1249-1256 (1981).

Other Reference Publication (61):
Bohnsack et al., "An IgA Monoclonal Antibody Directed Against Type III Antigen on Group B Streptococci Acts as an Opsonin," J. Immunol., 143:3338-3342 (1989).

Other Reference Publication (71):
D.E. Schiff et al., "Estimation of Protective Levels of Anti-O-Specific Lipopolysaccharide Immunoglobulin G Antibody Against Experiment Escherichia coli Infection," Infect. Immun., 61:975-980 (1993).

L2 ANSWER 37 OF 39 MEDLINE
AN 79018611 MEDLINE
DN 79018611 PubMed ID: 29470
TI Immunological properties of the lipopolysaccharide-protein complex of
Coxiella burnetii.
AU Kazar J; Schramek S; Brezina R
SO ACTA VIROLOGICA, (1978 Jul) 22 (4) 309-15.
Journal code: 0370401. ISSN: 0001-723X.
CY Czechoslovakia
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197811
ED Entered STN: 19900314
Last Updated on STN: 19950206
Entered Medline: 19781129
AB Purified lipopolysaccharide-protein complex (LPS-PC) extracted by
trichloroacetic acid from phase I Coxiella burnetii organisms induced in
mice and rabbits fair levels of **antibodies** directed to
antigen 1 and **antigen 2**, as detected by
complement-fixation (CF), microagglutination (MA), **opsonization-**
phagocytosis (OP) and serum protection (SP) tests. In guinea pigs
only very low levels of MA **antibodies** against **antigen**
2 were demonstrated. In rabbit serum, MA antibodies directed to
antigen 2 were found exclusively in the IgM fraction after the
primary immunizing dose; the second dose was followed by gradual shift of
MA antibodies to the IgG class. Two immunizing doses of the LPS-PC were
more effective when testing antibody response in mice or protection of
mice and guinea pigs against phase I virulent challenge.

L2 ANSWER 32 OF 39 MEDLINE
AN 80048686 MEDLINE
DN 80048686 PubMed ID: 91583
TI Immunological specificity of natural opsonins and their role in the
cross-reactivity between Staphylococcus aureus Mardi and Escherichia coli
101.
AU Young D A; Dobson P; Karakawa W W
SO INFECTION AND IMMUNITY, (1979 Sep) 25 (3) 954-9.
Journal code: 0246127. ISSN: 0019-9567.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198001
ED Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19800128
AB The immunochemical specificity of the observed cross-reactivity between
Escherichia coli strain 101 and Staphylococcus aureus strain Mardi was
examined. The cross-reactivity was shown to be dependent upon mucopeptide
antibodies which are present in normal and immune sera. Although both
organisms contained surface **antigens** with immunodominant
glucuronic acid residues, in vitro **phagocytosis** studies
indicated that **antibodies** directed against these
antigens were not significantly involved in the
opsonization process. Rather, **antibodies** with
mucopeptide specificity were shown to be involved in the in vitro
phagocytosis of these organisms by polymorphonuclear leukocytes.
The mucopeptide **antibodies**, which were found in both nonimmune
and immune sera, were shown to be effective in **opsonizing** both
the S. aureus strain and the E. coli strain. The ubiquitous distribution
of E. coli strains containing mucopeptide **antigens** common to
most bacteria suggests that these organisms may be responsible for the
wide prevalence of natural staphylococcal opsonins with mucopeptide
specificity in normal sera.

L2 ANSWER 29 OF 39 MEDLINE
 AN 81238589 MEDLINE
 DN 81238589 PubMed ID: 7019072
 TI Artificial Salmonella vaccines: Salmonella typhimurium O-antigen
 -specific oligosaccharide-protein conjugates elicit **opsonizing**
antibodies that enhance **phagocytosis**.
 AU Jorbeck H J; Svenson S B; Lindberg A A
 SO INFECTION AND IMMUNITY, (1981 May) 32 (2) 497-502.
 Journal code: 0246127. ISSN: 0019-9567.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 198109
 ED Entered STN: 19900316
 Last Updated on STN: 19900316
 Entered Medline: 19810915
 AB Outbred NMRI mice and rabbits were vaccinated with different artificial
 Salmonella typhimurium immunogens and the specificity and activity of
 elicited antibodies were studied in in vivo and in vitro phagocytosis
 assays. The Salmonella immunogens used were: (i) the synthetic
 disaccharide, albequose (formula see text) D-mannose, representative of
 Salmonella O antigen 4, covalently linked to bovine serum albumin (BSA);
 (ii) the octa- and dodecasaccharides, (formula see text) covalently linked
 to BSA; and (iii) whole heat-killed Salmonella. Rabbit antibodies
 passively administered to mice significantly enhanced the clearance of
 intravenously injected S. typhimurium challenge bacteria from the
 bloodstream. The clearance rate and the titer of anti-O-antigen-specific
 antibodies correlated. The clearance rate of an S. thompson (06,7) strain,
 which has a different O antigen, was the same irrespective of the rabbit
 serum given. NMRI mice actively immunized with the various
 oligosaccharide-BSA conjugates had a significantly increased clearance
 rate of S. typhimurium only. In the in vitro assay, mouse
 antioligosaccharide-BSA sera promoted phagocytosis of S. typhimurium, but
 not S. thompson, when incubated with complement and mouse peritoneal
 exudate cells activated with Freund complete adjuvant.

L2 ANSWER 19 OF 39 MEDLINE
 AN 88189327 MEDLINE
 DN 88189327 PubMed ID: 3258649
 TI The binding site for Clq on IgG.
 AU Duncan A R; Winter G
 CS MRC Laboratory of Molecular Biology, Cambridge, UK.
 SO NATURE, (1988 Apr 21) 332 (6166) 738-40.
 Journal code: 0410462. ISSN: 0028-0836.
 CY ENGLAND: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 198805
 ED Entered STN: 19900308
 Last Updated on STN: 19900308
 Entered Medline: 19880519
 AB In humoral defence, pathogens are **cleared by antibodies** acting as adaptor molecules: they bind to **antigen** and trigger **clearance** mechanisms such as **phagocytosis**, **antibody**-dependent cell-mediated cytotoxicity and complement lysis. The first step in the complement cascade is the binding of Clq to the antibody. There are six heads on Clq, connected by collagen-like stems to a central stalk, and the isolated heads bind to the Fc portion of antibody rather weakly, with an affinity of 100 microm (ref. 3). Binding of antibody to multiple epitopes on an antigenic surface, aggregates the antibody and this facilitates the binding of several Clq heads, leading to an enhanced affinity of about 10 nM (ref. 1). Within the Fc portion of the antibody, Clq binds to the CH2 domain. The interaction is sensitive to ionic strength, and appears to be highly conserved throughout evolution as Clq reacts with IgG from different species (for example see ref. 8). By systematically altering surface residues in the mouse IgG2b isotype, we have localized the binding site for Clq to three side chains, Glu 318, Lys 320 and Lys 322. These residues are relatively conserved in other antibody isotypes, and a peptide mimic of this sequence is able to inhibit complement lysis. We propose that this sequence motif forms a common core in the interactions of IgG and Clq.

L2 ANSWER 8 OF 39 MEDLINE
 AN 1998286564 MEDLINE
 DN 98286564 PubMed ID: 9623361
 TI Intravenous immunoglobulin preparations as immunomodulatory agents.
 AU Dimitrijevic M; Popovic M; Stefanovic D; Petronijevic M
 CS University School of Pharmacy, Belgrade.
 SO VOJNOSANITETSKI PREGLED, (1998 Mar-Apr) 55 (2 Suppl) 63-9.
 Journal code: 21530700R. ISSN: 0042-8450.
 CY Yugoslavia
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199808
 ED Entered STN: 19980903
 Last Updated on STN: 19980903
 Entered Medline: 19980824
 AB Certain immunopathologic conditions, such as hypersensitivity and autoimmune diseases, are characterized by quantitative and/or qualitative alterations of immune reactions. It is now believed that such immunologic disturbances generate from inadequate internal control and regulation of immunologic reactivity. Important member of the complex regulatory network that supervises an immune response are antibodies themselves. Since antibody is bifunctional molecule, its regulatory action deals with two separate molecular structures denoted as Fab and Fc portion. By Fab fragment, antibody interferes with the reaction with **antigens**, or participates in the regulatory idiotype/antiidiotype interactions. Fc-mediated regulation includes influence on complement activation cascade, formation and **clearance** of immune complexes, **phagocytosis**, ADCC activity, T- and B-cell function, cytokine profile, etc. In general, **antibody** triggers immune reactions, but also has the capacity to suppress them. Exogenous antibodies are likely to elicit similar effects on immune processes. Actually, it has been demonstrated that intravenously given immunoglobulins, particularly high-dose IgG, effectively combat harmful immune response in some chronic inflammatory and autoimmune disorders. Hence, preparations of immunoglobulins for intravenous use (IVIG) can be considered as an immunomodulatory agent. To achieve the property of modulating the immune response, IVIG products must contain intact (7S) IgG molecule and maintain sufficient concentrations in plasma.

L2 ANSWER 4 OF 39 MEDLINE
 AN 2000231768 MEDLINE
 DN 20231768 PubMed ID: 10768923
 TI Binding to and opsonophagocytic activity of O-antigen-specific monoclonal antibodies against encapsulated and nonencapsulated *Klebsiella pneumoniae* serotype O1 strains.
 AU Held T K; Jendrike N R; Rukavina T; Podschun R; Trautmann M
 CS Department of Hematology and Oncology, Charite/Campus Virchow-Klinikum, Humboldt University, 13353 Berlin, Germany.
 SO INFECTION AND IMMUNITY, (2000 May) 68 (5) 2402-9.
 Journal code: 0246127. ISSN: 0019-9567.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 200006
 ED Entered STN: 20000622
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 AB The high mortality of nosocomial infections caused by *Klebsiella* spp. has acted as a stimulus to develop immunotherapeutic approaches targeted against surface molecules of these bacteria. Since O-antigen-specific antibodies may add to the protective effect of K antisera, we tested the functional and binding capacity of O-antigen-specific monoclonal antibodies (MAbs) raised against different *Klebsiella* O antigens. The MAbs tested were specific for the O-polysaccharide partial antigens D-galactan II (Mab Ru-O1), D-galactan I (Mab IV/4-5), or core oligosaccharide (Mab V/9-5) of the *Klebsiella* serogroup O1 antigen. In enzyme-linked immunosorbent assay binding experiments, we found that all MAbs recognized their epitopes on intact capsule-free bacteria; however, binding to encapsulated wild-type strains belonging to different K-antigen serotypes was significantly reduced. The K2 **antigen** acted as the strongest penetration barrier, while the K7 and K21 **antigens** allowed some, though diminished, **antibody** binding. In vitro **phagocytic** killing experiments showed that Mab Ru-O1 possessed significant **opsonizing** activity for nonencapsulated O1 serogroup strains and also, to a much lesser extent, for encapsulated strains belonging to the O1:K7 and O1:K21 serotypes. MAbs or antisera specific for the D-galactan II antigen may thus be the most promising agents for further efforts to develop a second-generation *Klebsiella* hyperimmune globulin comprising both K- and O-antigen specificities.